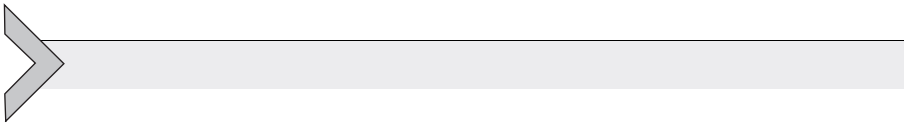
Sports, Exercise, and Nutritional Genomics

Current Status and Future Directions

Edited by Debmalya Barh and Ildus I. Ahmetov



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Sports genetics

CHAPTER ONE

Introduction to genetics of sport and exercise

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1.1 Nature vs nurture influence

It is acceptable to assume that any individual who is highly committed and dedicated to physical training is able to improve performance provided that the stimulus is adequate. However, some individuals appear to be naturally gifted with superior baseline traits or better responses to training than others (Tucker and Collins, 2012). It has been common to observe in the scientific literature a large interindividual variance in the response to training in several phenotypes relevant to physical performance (Bouchard 2012). Even among elite athletes, there may be a wide phenotypic variation—the curve distribution of individual performances for a given sport discipline will certainly show a normal distribution with a limited number of individuals at the extremities. Few individuals seem to be exceptionally gifted and demonstrate extraordinarily high-performance levels. A number of variables can contribute to explain this interindividual variance in response to training (Mann et al., 2014). Human physical performance expresses at a given time point a complex phenomenon, which is the result of the interaction of numerous intrinsic and extrinsic factors (Guilherme et al., 2014). Indeed, many of the intrinsic factors known to contribute to performance-related traits are within their own complex phenotypes determined by both nature and nurture (Tucker and Collins, 2012).

The relative contributions of genetics and environment to phenotypic variation in numerous behavioral and biological traits have long been a topic of interest (Bouchard and Malina, 2014). The degree to which performance potential is predetermined by inherited traits against the degree influenced by environmental factors (training, nutrition, motivation, development opportunities, and overall health conditions) has excited much debate, that is often framed as "nature *vs* nurture" (Gibson, 2016). The terms nature and nurture were first used in the late 19th century (Galton, 1875). Based on twins and pedigree records, Francis Galton proposed that there is no escape from the conclusion that nature prevails enormously over nurture when the

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differences of nurture do not exceed what is commonly to be found among persons of the same rank of society and in the same country (Galton, 2012). This implies that the upper limit for an individual performance among persons exposed to the same environment is predetermined by heritable characteristics which no amount of practice or other natural environmental factor could overcome. However, there is also evidence that environmental factors have remarkable influence on performance (Davids and Baker, 2007).

Most of the understanding about environmental influences on exercise performance comes from social and psychological research, which claims that performance in a given task is learned and achieved through extended practice of a given skill (Yan et al., 2016). In 1993, it was proposed that when individuals engage in practice activities with full concentration on improving some specific aspect of performance, the so-called deliberate practice, there is a linear improvement of an individual performance through repetition and successive refinement (Ericsson et al., 1993, 2009). Thus, as a consequence of the accumulated deliberate practice, performance in many areas of expertise considerably increases and can bring out the best in you. In fact, top-level athletes accumulate years of a rigid and organized training schedule on the road to sports excellence, in which quality rather than the amount of training can differentiate athletes of different competitive levels (Ericsson, 2013). Moreover, it was proposed that expert (i.e., top-level) athletes accumulate more hours of training than nonexperts; in particular, they devote more time in activities deemed most relevant for their respective disciplines (Davids and Baker, 2007). Therefore, the deliberate practice appears to be an important contributing factor to performance achievement, but it is not the only one.

A recent metaanalysis showed that deliberate practice accounted for only 18% of the variance in sports performance, leaving 82% of the variance explained by other factors (Macnamara et al., 2016). In fact, the environment influence on sports performance also includes motivation, family support, coaches' influence, local culture, relative age effect, and birthplace effect (Davids and Baker, 2007; Yan et al., 2016). All these environmental factors are undoubtedly critical to performance achievement, but the current literature does not support the idea that interindividual variance in sports performance is mainly due to environmental factors (Georgiades et al., 2017). Heritability studies have provided evidence that performance phenotypes are substantially inherited, since all functional capacities, morphological dimensions, motor attributes, personality traits, and cognitive abilities showed moderate to high heritability (Georgiades et al., 2017). In this sense, the current accumulated literature tips the balance in favor of the relative contribution of genetics (Georgiades et al., 2017); however, the genetic basis of performance phenotypes is still under intense investigation. Particularly, the understanding of the influence of genetics on physical performance have been challenging due to its complex nature (Guilherme et al., 2014).

Two main classes of studies evaluating the genetic basis of performance phenotypes were conducted in the course of history. The first class was designed to explore genetic

epidemiology paradigms, focusing on the magnitude of the genetic contribution for a relevant phenotype and characteristics of inheritance patterns. These studies dominated the landscape of exercise genetics research from 1960s to the early 1990s, and were based on twins or members of nuclear families (Bouchard and Malina, 2014). The second class was designed to identify locus, genes, and DNA sequence variants contributing to phenotypic variation. For this, several research designs and technologies were used over the past two decades, such as: case-control association studies, genome-wide linkage scans, and genome-wide association studies (Bouchard and Malina, 2014). More recently, analyses of "omics" approaches (e.g., genomics and transcriptomics) have been introduced for the evaluation of performance phenotypes (Sarzynski et al., 2015). Based on these studies, more than 200 genetic markers have been shown to be associated with a performance-relevant trait in at least one study (Ahmetov et al., 2016; Bray et al., 2009). Of note, a genetic marker is a DNA sequence whose address is known and that can be used as a reference point in the human genome.

Collective information of DNA markers has been used for the construction of genetic maps (Bray et al., 2009), allowing the identification of variants contributing to several phenotypic variations observed in human physical performance. The human gene map for performance-related traits continues to grow over the years (Ahmetov et al., 2016; Sarzynski et al., 2016). In the following sections of this introductory chapter, we will address brief historical and basic concepts, evidence on genetic contributions to performance phenotypes, main methods of investigation used, and future research perspectives.

1.2 Genetics and olympic glory: early studies on olympic athletes

The first attempt to identify differences in genetic markers for performance-related traits dates to the late 1960s (Bouchard and Malina, 2014). Due to their uniquely high levels of performance, elite athletes are considered to be predisposed for improving motor performance. In this sense, taking advantage of the 1968 Olympic Games in Mexico, an international group of researchers investigated 1265 competitors of both genders (although only 148 were women) and from almost all events in order to discriminate the body shape and the distribution of blood groups of Olympic athletes (de Garay et al., 1974). The study evaluated athletes of different ethnicities, sport disciplines, and competitive levels, however, at that time the methodologies available for the study of genetics were limited, and thus the expected conclusions were also limited. The authors addressed the inheritance of athleticism by estimating phenylthiocarbamide (PTC) threshold, red blood cell (RBC) antigens and enzymes, as well as chromosome patterns (karyotype). The PTC threshold was underrepresented among the athletes, and no woman with the XY chromosome was found, nor men with the XYY pattern occurred

among the 15 women and 227 men karyotyped (Tanner, 1976). Unfortunately, most of the findings were inconclusive (Evans Robson, 1977; Tanner, 1976).

The effort to evaluate genetic characteristics of Olympic athletes was continued on the occasion of the 1976 Olympic Games in Montreal (Chagnon et al., 1984; Couture et al., 1986). In these studies, an attempt to associate genetic variants with performance was done by exploring RBC antigens and enzymes in a subsample of Caucasian athletes ($n \leq 79$ individuals) who participated in endurance sports (track and field, canoeing, cycling, swimming, or rowing). Gene frequencies were reported and no significant differences were observed between athletes (i.e., cases) and a nonathletic reference population (i.e., controls). Taken together, none of these initial studies identified reliable and predictive genetic markers of superior physical performance (Bouchard and Malina, 2014).

Among early studies, the most compelling data relating a genetic variant to superior athletic performance is the case of an Olympic gold medalist in cross-country skiing (Longmore, 1993). In the early 1990s, some members of a large family from Northern Europe were examined with naturally high RBC levels, although the cause of this erythrocytosis was not clear at the time (Juvonen et al., 1991). After a deeper evaluation, it was demonstrated that erythrocytosis is segregating in this family, and as a consequence, these individuals present familial erythrocytosis—an inherited condition characterized by a very high number of RBC (Juvonen et al., 1991). The aforementioned athlete is a member of this family and was evaluated along with other relatives. At the time of writing these studies, the athlete was 53 years old and has always been in excellent health, but had elevated hemoglobin levels (20 g/dL or greater) since childhood. Of note, he was one of the best cross-country skiers in the world, having won three Olympic gold medals, two Olympic silver medals, two bronze Olympic silver medals, besides five medals in World championships (competitions at distances between 10 and 30 km).

The impact of erythrocytosis on the health of the affected members of this family in uncertain, but it seems to have caused few problems in them, being compatible with long life, good health, and exceptional physical fitness—probably due to an increased sensitivity to erythropoietin (Juvonen et al., 1991). Has been shown that the affected individuals did not present altered serum erythropoietin concentration; however, these individuals may present high erythrocytosis even at low concentrations of erythropoietin (Juvonen et al., 1991). The resulting high hemoglobin concentration leads to an increased oxygen-carrying capacity, potentially improving endurance performance since a high aerobic capacity is a result of an outstanding maximal cardiac output and a high oxygen-carrying capacity, together leading to a high level of maximal oxygen uptake and an optimal oxygen delivery to the exercising muscles (Lundby and Robach, 2015).

Familial erythrocytosis has been reported to be a dominant inheritance trait that shows linkage to a polymorphic locus adjacent to the erythropoietin receptor (*EPOR*) gene (de la Chapelle et al., 1993a). Therefore, using genomic regions deposited in the

GenBank database, a group of researchers designed primers to amplify the 3′ region of the *EPOR* gene comprising exons 7 and 8 and the intron between them (de la Chapelle et al., 1993b). Sequencing of the region amplified revealed a G to A mutation at position 6002 in affected individuals, which alters for amino acid 439 of the EPOR protein from TGG encoding tryptophan to TAG (a stop codon); as a consequence, the resulting protein product is truncated for all 70 amino acids downstream of the mutation (de la Chapelle et al., 1993b). A total of 29 members of the family evaluated were classified as affected, and all 29 were carriers of the mutation (i.e., carriers of the heterozygous genotype); conversely, none of 21 clinically unaffected individuals had the mutation (de la Chapelle et al., 1993b). Thus, possessing only one allele of the mutation is sufficient to bring about a phenotypic effect (i.e., familial erythrocytosis). Other mutations cannot be formally excluded, but the G to A mutation showed an important biological relevance.

It is noteworthy that familial forms of erythrocytosis are rare, and therefore, although the affected individuals of this family (with an Olympic champion among them) might possess an inherent advantage in aerobic-oriented events, few individuals around the world are carriers of this natural "advantage," especially under a favorable environmental condition. The remaining question is which other variants (whether rare or not) could predispose an individual to a better trainability of performance phenotypes.

1.3 The phenomenon of athletes from west and east africa

An intriguing question in Sports Science is the dominance of specific population groups at the opposite ends of the competitive running spectrum (see Tables 1.1 and 1.2).

Table 1.1 Men outdoor world records.

Discipline	Athlete	Perf.	Date	Country	Ethnicity
100 m	Usain Bolt	9.58	Aug. 16, 2009	Jamaica	African-American
100 m hurdles	Aries Merritt	12.80	Sep. 7, 2012	United States	African-American
200 m	Usain Bolt	19.19	Aug. 20, 2009	Jamaica	African-American
1500 m	Hicham El Guerrouj	3:26.00	Jul. 14, 1998	Morocco	North Africa
3000 m	Daniel Komen	7:20.67	Sep. 1, 1996	Kenya	East Africa
5000 m	Kenenisa Bekele	12:37.35	May 31, 2004	Ethiopia	East Africa
10,000 m	Kenenisa Bekele	26:17.53	Aug. 26, 2005	Ethiopia	East Africa
Half marathon	Abraham Kiptum	58:18	Oct. 28, 2018	Kenya	East Africa
Marathon	Eliud Kipchoge	02:01:39	Sep. 16, 2018	Kenya	East Africa

This table is based on information from the International Association of Athletics Federations (http://iaaf.org).
Perf., performance.

Table 1.2 Women outdoor world records.

Discipline	Athlete	Perf.	Date	Country	Ethnicity
100 m	Florence Griffith-Joyner	10.49	Jul. 16, 1988	United States	African-American
100 m hurdles	Kendra Harrison	12.20	Jul. 22, 2016	United States	African-American
200 m	Florence Griffith-Joyner	21.34	Sep. 29, 1988	United States	African-American
1500 m	Genzebe Dibaba	3:50.07	Jul. 17, 2015	Ethiopia	East Africa
3000 m[a]	Genzebe Dibaba	8:16.60	Feb. 6, 2014	Ethiopia	East Africa
5000 m	Tirunesh Dibaba	14:11.15	Jun. 6, 2008	Ethiopia	East Africa
10,000 m	Almaz Ayana	29:17.45	Aug. 12, 2016	Ethiopia	East Africa
Half marathon	Netsanet Gudeta	01:06:11	Mar. 24, 2018	Ethiopia	East Africa
Marathon	Mary Jepkosgei Keitany	02:17:01	Apr. 23, 2017	Kenya	East Africa

[a]Indoor performance.
This table is based on information from the International Association of Athletics Federations (http://iaaf.org). *Perf.*, performance.

There is a distinct geographical concentration of success between sprint running events and distance running events. In sprint/power-oriented running events, there is a dominance of African-American athletes born in the United States or Caribbean Islands, especially Jamaica. In particular, most of these athletes are African-American with earlier ancestry from West Africa (Pitsiladis, 2011; Tucker et al., 2013). At the opposite end of the spectrum, that is, endurance events, we observed a dominance of athletes from Kenya and Ethiopia (i.e., of East African descent). Of the all-time top-50 male athletes in the Marathon, all come from East Africa; moreover, of the all-time top-100 male marathon runners, 92 come from East Africa (IAAF, 2018). Similarly, there is a dominance of the athletes from East African descent among female marathon runners (IAAF, 2018).

Among the world's best runners (between years 1999 and 2015), most male runners in the 10 km, half-marathon and marathon were Kenyans; similarly, most female runners in the 10 km and half-marathon were Kenyans (for the marathon, there was a similar proportion of Kenyans and Ethiopians) (Nikolaidis et al., 2017). The dominance of Kenya in distance running is increased by the observation that a large majority of Kenya's most successful runners originate from a single tribe, the Kalenjin (Tucker et al., 2013). Questionnaires administered to 404 elite Kenyan middle-distance/distance runners (i.e., from the 800 m to the marathon) revealed that most of these athletes came from the Rift Valley province, where the Kalenjin ethnic group lives; in particular, a considerable proportion of these athletes come from a subtribe of the Kalenjin known as the Nandi (Onywera et al., 2006). This information highlights that individuals who are born in particular places share genetic or environmental factors that may predispose them to success in a given

sport. The underlying factors enabling this concentrated performance achievement have been the subject of considerable research, and theories emerged that West and East Africans are genetically predisposed to sprint and distance running, respectively (Tucker et al., 2013). Although genetics might not be the unique determinant of population differences on performance, evidence suggests an important role of genetics (Pitsiladis and Scott, 2005).

In specific populations, there may be selection for a particular phenotype such as superior sprint or endurance performance, if it offers a selective advantage in that environment (Pitsiladis, 2011). In this case, the genetic background beneficial to sprint or endurance can prevail and predispose the population for the type of favorable performance. For example, variations in somatotype and genetic patterns in muscle fiber characteristics, biochemical metabolic pathways or pulmonary physiology which result in favorable biological consequences for sprinting may have been concentrated by natural selection over the centuries in the Afrocentric peoples and displaced from West Africa to the New World during the slave trade (Morrison and Cooper, 2006). Alternatively, it was hypothesized that only the strongest slaves survived to the harsh living conditions of the displacement process from West African across the Atlantic (Pitsiladis, 2011). Although these hypotheses remain without adequate scientific proof, it has been postulated that superior sprint performances of African-Americans of primarily West African origin were due to favorable sprint biology. Conversely, it was hypothesized that the Nandi tribe in Kenya has been concentrated by selection over the centuries for endurance performance through cultural practices such as cattle theft, also known as cattle raiding (Manners, 1997). These hypotheses have potential theoretical underpinnings, but the genetic background responsible for the superior performance of African-Americans (or West Africans) and East Africans still needs to be identified.

The first genetic approach conducted in African athletes was to investigate the genetic ancestry of Ethiopian and Kenyan distance runners using the uniparentally inherited genetic marker: the mitochondrial DNA (mtDNA) (Scott et al., 2009, 2005b). Typically, mtDNA is inherited matrilinearly (i.e., from mother to child), undergoing no recombination and only changing with the rise of new mutations (Pitsiladis, 2011). This results in the accumulation of linked complexes of DNA variants (i.e., "haplogroups" or "haplotypes") down different lines of descent from an ancestral mtDNA molecule, which can be used to trace the ancestry of individuals or populations (Scott et al., 2005b). Haplotype groups represent easily comparable units of genealogical information, that when found in other populations can be used as indicators of recent migrations. The studies conducted in Ethiopian and Kenyan athletes, revealed a wide distribution of mtDNA haplogroups, in contrast to the concept that these individuals are a genetically distinct group as defined by mtDNA, that is, these individuals did not remain isolated in East African but shows that there were migration events and subsequent admixture (Wilber and Pitsiladis, 2012). The theory that the elite East African runners do not arise

from a limited genetic isolate is further supported by the analysis of the Y chromosome haplogroups distribution in Ethiopians (Moran et al., 2004). Of note, the Y chromosome can be considered the male equivalent of mtDNA (Wilber and Pitsiladis, 2012).

Apart from their role on the ancestry of individuals or populations, variants in mtDNA have been suggested to influence the variance in human physical performance as mtDNA encodes various subunits of enzyme complexes of oxidative phosphorylation, as well as components of the mitochondrial protein synthesis system (Pitsiladis, 2011). In context, although elite East African runners are not a mitochondrially distinct group (Scott et al., 2005b), international- and national-level runners from Kenya showed differences in mtDNA haplogroups distribution in comparison to the general Kenyan population (Scott et al., 2009). These findings suggest that mtDNA haplogroups contain DNA variants that can influence some aspects of endurance performance or its trainability, but it may not fully explain the Kenyan running performance (Wilber and Pitsiladis, 2012). Indeed, variants in the nuclear genome and environment factors can also be determinants of East African running success.

Two variants in the nuclear genome have been widely studied in sports and exercise: the angiotensin-converting enzyme (*ACE*) I/D and the α-actinin-3 (*ACTN3*) R577X gene variants. The first is an insertion (I) or deletion (D) allele of 287 base pairs in intron 16 of the *ACE* gene (Puthucheary et al., 2011), which has been estimated to explain up to 47% of the variance in circulating ACE levels (Rigat et al., 1990)—an enzyme that cleaves the circulating angiotensin-I giving rise to a biologically highly active oligopeptide, the so-called angiotensin-II (Puthucheary et al., 2011). The I-allele might provide a potential advantage to endurance-oriented events, whereas the D-allele might benefit performance in strength/power-oriented events (Myerson et al., 1999). The second is a C to T mutation at position 1747 in exon 16, which alters for amino acid 577 of the α-actinin-3 protein from CGA encoding an arginine to TGA (a stop codon); as a consequence, the resulting protein product is truncated for all amino acids downstream of the mutation (North et al., 1999). The α-actinins are important structural proteins of skeletal muscle. In human skeletal muscles, there are two genes encoding α-actinins: *ACTN2*, which is expressed in all fibers and *ACTN3*, which is restricted to fast fibers (i.e., type II fibers) (North and Beggs, 1996). It has been proposed that the X-allele (i.e., related to the truncated protein) can provide an advantage to endurance-oriented events, whereas the R-allele (i.e., related to the normal protein translation) can be an advantage to strength/power-oriented events (Yang et al., 2003).

Both gene variants (i.e., *ACE* I/D and *ACTN3* R577X) were evaluated in East and West African athletes, however, no significant association between the variants and athlete status was found (Ash et al., 2011; Scott et al., 2005a, 2010; Yang et al., 2007). In summary, genotyping two of the key candidate genes for human physical performance in highly successful athletes revealed that these genes should not be expressive determinants of their success. Whether other nuclear variants can help to explain such

phenomena remains to be determined, however, the extraordinary performance achievement must rely on the successful integration of a number of physiological, biochemical, and biomechanical systems, which themselves are the product of a combination of variables (Wilber and Pitsiladis, 2012). Of note, altitude, training-related aspects, habitual diet, cultural aspects, and socioeconomic factors are also contributors of sports success (Tucker et al., 2013).

1.4 The human genome variation: basic concepts

The complete set of genetic information that an organism possesses embodied in its complete DNA sequence is called genome. The genome carries the information for all the proteins and RNA molecules that the organism will ever synthesize, as well as products participating in the regulation of gene expression and other cellular events. The Human Genome Project, an international collaboration which operated from 1990 to 2003, worked to provide researchers with basic information about the sequences of the human genome. With the availability of the draft sequences providing a first overall view of the human genome in 2001 (Lander et al., 2001; Venter et al., 2001) and later the availability of the near-complete sequence of the human genome (International Human Genome Sequencing Consortium, 2004), one could begin to look for deviations from the reference sequence and to begin exploring in-depth interindividual differences at the DNA level (Bouchard, 2015). Nowadays, the exploration of interindividual differences at the DNA level was greatly facilitated by other major projects subsequent to the Human Genome Project, such as the International HapMap Project, the 1000 Genome Project and similar consortia. These consortia are intended to map the patterns of genetic diversity in the human genome, and consequently, establish a reference for genetic association studies. For example, the 1000 Genomes Project, which operated from 2008 to 2015, generated a data set that contains data of 2504 individuals from 26 populations, so creating the largest public catalog of human genetic diversity and genotype data (Sudmant et al., 2015). For researchers, a catalog of human genetic variations of literally thousands of individuals available for further detailed analysis can be considered an important advance for science, because then we have a comparative baseline.

Sequencing the human genome has revealed that the DNA sequence is more than 99% identical among individuals, and therefore, a small fraction of the human genome (~0.1%) may be responsible for the wide phenotypic diversity observed in the population (International Human Genome Sequencing Consortium, 2004). Due to the all-size of the human genome, this small fraction can represent millions of base pairs that differ in the DNA sequence between individuals. Although most of these DNA variants appear to have little or no functional consequence, a set of these DNA differences may reflect the normal phenotypic variation observed in humans, including differences in anatomy, physiology, personality traits, and athletic ability (Guilherme et al., 2014). Therefore,

the aim of human genetics research is to identify specific DNA differences that are influencing a given phenotype.

Performance phenotypes (e.g., maximal strength, sprinting, movement economy, and maximum oxygen uptake) are very complex traits, influenced by several genes on different tissues and organs and multiple nongenetic environmental factors (Guilherme et al., 2014). Regarding the genetic component, relevant phenotypes can be influenced by small-scale sequence changes in either the regulatory region of a gene or the DNA coding sequence. The simplest and most abundant type of genetic variation found in the human genome is the single-nucleotide polymorphism (SNP). About one nucleotide in every 200–300 of the DNA sequence is polymorphic (i.e., more than one form is common in the population) (Salisbury et al., 2003). The word polymorphism is used by molecular geneticists to describe a variant having a frequency in the population above 1%.

Although other types of variant coexist in the human genome and can impact complex traits, SNPs are largely the easiest to ascertain and the most useful and widely applied markers in human genetic studies (Johnson, 2009). The DNA is encoded by four different nucleotides [adenine (A), thymine (T), cytosine (C), and guanidine (G)] that, by binding in sequence, make a single DNA strand sequence (e.g., …AACGGT…) (Guilherme et al., 2014). SNPs consist of a change in a single nucleotide of the DNA sequence (Salisbury et al., 2003). For example, in the hypothetical sequence "AAC*G*GT" the nucleotide G was identified as polymorphic, and some individuals have a nucleotide A rather than G, so the alternative sequence is "AAC*A*GT." The different sequences that a particular polymorphism may take are called alleles. Given the very large number of SNPs, these variants are designated by numbers beginning with "rs" (e.g., rs1815739) and cataloged in the public dbSNP database (the NCBI database of SNPs and multiple small-scale variants).

In DNA coding sequences, each sequence of three nucleotides (called codons) encodes for one specific amino acid in the peptide chain of the final gene product (Guilherme et al., 2014). Therefore, if the SNP occurs in DNA coding sequences, the final gene product can be affected, depending on the type of variant: synonymous, missense, or nonsense (Table 1.3). Synonymous or silent SNPs do not change an amino acid in the final gene product; in contrast, nonsynonymous SNPs result in an altered codon that specifies either a different amino acid (a missense variant) or a termination codon (a nonsense variant) (Bouchard et al., 2011a). A missense variant can induce either a conservative or nonconservative amino acid substitution. While a conservative missense SNP does not actually change the chemical properties of the molecule, the amino acid introduced by a nonconservative missense SNP has different chemical characteristics (Bouchard et al., 2011a). For instance, a nonconservative missense SNP can affect the shape of the final gene product. Regarding the nonsense variants, a classic example is the aforementioned *ACTN3* R577X polymorphism.

Table 1.3 The main classes of small-scale genetic variants.

Variant type	Alleles	Coding sequence (CDS)	Encoded amino acids
Synonymous	Common allele	…ATC.TT**A**.AAA…	…Ile.**Leu**.Lys…
	Alternative allele	…ATC.TT**G**.AAA…	…Ile.**Leu**.Lys…
Missense	Common allele	…ATC.TT**A**.AAA…	…Ile.**Leu**.Lys…
	Alternative allele	…ATC.TT**T**.AAA…	…Ile.**Phe**.Lys…
Nonsense	Common allele	…ATC.T**T**A.AAA…	…Ile.**Leu**.Lys…
	Alternative allele	…ATC.T**A**A.AAA…	…Ile.Stop.—
Short insertion	Common sequence	…ATC.TCT.CAA …	…Ile.Ser.Gln…
	Alternative sequence	…ATC.**CA**T.CTC…	…Ile.**His**.**Leu**…
Short deletion	Common sequence	…ATC.**T**CT.CAA …	…Ile.Ser.Gln…
	Alternative sequence	…ATC.CTC.AAG…	…Ile.**Leu**.**Lys**…

Gln, glutamine; *His*, histidine; *Ile*, isoleucine; *Leu*, leucine; *Lys*, lysine; *Phe*, phenylalanine; *Ser*, serine.

The second most common class of polymorphism found in the human genome is the insertions/deletions (indels) of one or a few nucleotides (Table 1.3). These variants may introduce frameshifts, ending the normal chain of amino acids and replacing the remainder with a different chain of amino acids (Gibson, 2016). Polymorphisms used to be viewed as functionally significant only if they affected the DNA coding sequence, however, variants in the noncoding sequence may also have significant effects on gene regulation (Bouchard et al., 2011a).

Although ~99.9% of variants consist of SNPs and short indels, other types of variants, including microsatellites, copy number variants, large indels, and epigenetic markers may also be important for phenotypic variation (Auton et al., 2015).

1.5 Research methodologies in genetics of sport and exercise

Initial studies aimed at defining the genetics of human physical performance were essentially observational based on the pairs of twins (i.e., twin-studies) or members of nuclear families (i.e., familial aggregation studies), and focused on estimating the heritability of different traits of interest (Bouchard and Malina, 2014). In general, these studies have shown that there was less variance among more genetically related individuals (e.g., within pairs of monozygotic twins) than in less genetically related individuals, suggesting an important contribution of genetics to performance-related traits (Bouchard et al., 2011a). For example, Prud'homme et al. (1984) submitted 10 pairs of monozygotic twins to a 20-week endurance-training program and found that trainability of maximal aerobic power is largely influenced by heritability. The intraclass correlation coefficient computed from the amount of improvement in maximal aerobic performance (mL O_2/min/kg) induced by training reached 0.74 ($P < 0.01$), indicating that monozygotic twin pairs yielded approximately the same responsiveness to training

(Prud'homme et al., 1984). Thereafter, findings from the classical HERITAGE family study also indicated based on the nuclear families an important heritability estimate of the maximum oxygen uptake ($\dot{V}O_{2max}$) (Bouchard et al. 1999). Following the 20-week endurance training program, a variance in $\dot{V}O_{2max}$ response (mL O_2/min) between individuals was observed, however, there were 2.5 times more variance ($P = 0.0001$) between than within families, showing that the $\dot{V}O_{2max}$ response to training aggregates in families (Bouchard et al., 1999). Muscle strength and mass phenotypes have also been quantified in twin and family studies, and the results also indicated a relatively high heritability estimates (Thomis and Aerssens, 2012).

Although unquestionably important, these studies do not return information about which genes and gene variants are contributing to the relevant phenotypes (Guilherme et al., 2014). However, with the recent development of more advanced molecular biology techniques, genetic studies are no longer restricted exclusively to family studies but have been expanded to include the evaluation of locus, genes, and DNA sequence variants within populations or between distinct athletic groups (Pitsiladis and Wang, 2011). Over the past 20 years, there has been a great effort to identify at the DNA-level polymorphisms that may contribute to performance phenotypes. The most frequent experimental approach used during this period was the genetic association study between a candidate polymorphism and relevant phenotypes. These studies can be divided into three main categories: (1) case-control association studies, which compare the frequency of a particular polymorphism (i.e., specific genotypes and their alleles) in a cohort of cases (individuals with the trait of interest) and controls (individuals without the trait of interest); (2) cross-sectional association studies, which compare selected physiological and/or performance data between specific genotype groups; and (3) longitudinal association studies, in which responses to a given intervention (e.g., exercise training or diet intervention) are compared between genotype groups (Guilherme et al., 2014). Of these, case-control association design remains the most common approach in sports and exercise genetics studies (Ahmetov and Fedotovskaya, 2015), mainly because they have a relatively low cost and are straightforward to do, which makes this approach interesting to evaluate potential candidate polymorphisms in different populations or cohorts.

In a case-control association study, for example, if one genotype or allele of a given polymorphism is more frequent in elite athletes (i.e., the "cases") than in their nonathletic referents (i.e., the "controls"; usually adult nonathletes), thus an association between the polymorphism and the athletes can be proposed, but not a cause-effect relationship (Lucia et al., 2010). In order to better establish a physiological or rationale role between the variants and relevant phenotypes, cross-sectional and longitudinal approaches are necessary. For example, the first polymorphism associated with human physical performance (i.e., the *ACE* I/D polymorphism) was identified in 1998 (Gayagay et al., 1998; Montgomery et al., 1998). In the first two published papers about the *ACE* I/D polymorphism, elite British mountaineers (Montgomery et al., 1998) and Australian national

rowers (Gayagay et al., 1998) were genotyped and had the polymorphism frequency compared with nonathletic referents—a classical case-control association study. In both studies, it was found a higher frequency of the I-allele among athletes, that is, an association was found between the polymorphism and athletic status. Therefore, a possible relevance of this variant for elite endurance athletes was proposed, but not a cause-effect relationship.

Regarding the *ACE* I/D polymorphism, it was shown that the D-allele (i.e., deletion allele) rather than the I-allele (i.e., insertion allele) allele is associated with higher circulating ACE levels in healthy Caucasians (Rigat et al., 1990) and elite Ironman triathletes (Domingo et al., 2013). Moreover, there was a positive correlation between plasma ACE activity and ACE protein concentration, and a positive association between plasma ACE activity and overall finishing time within the South African-born participants who completed the 2000 and/or 2001 South African Ironman Triathlon in under 15 h (Domingo et al., 2013). Furthermore, based on a muscle biopsy study, it was suggested that I-allele carriers (seven healthy nonathletic individuals) may have an amplified adjustment of myocellular organelles and gene transcripts related to mitochondrial lipid metabolism after a bicycle endurance training (Vaughan et al., 2013). Although a more solid conclusion is still lacking, these are examples of attempts aimed to establish a cause-effect relationship between the polymorphism and relevant phenotypes. Similar attempts have also been explored for other polymorphisms, such as the *ACTN3* R577X variant (Vincent et al., 2007, 2012).

One major disadvantage of these association studies with candidate polymorphisms is that only one or few gene variants can be assessed at a time. To improve the search for relevant variants, new methods exploring a greater extent of the human genome were developed. In this sense, genome-wide linkage study (GWLS) was the first approach proposed (Bouchard et al., 2011a). GWLS identifies chromosomal regions that harbor genes affecting quantitative traits over generations, and therefore, requires familial data not. The identification of these chromosomal regions, referred to as quantitative trait loci (QTL), is the first step in attempt to associate genetic variations with relevant phenotypes. Because a typical QTL may span millions of DNA base pairs and a large number of genes, progressing from the QTL to the causal DNA sequence and alleles requires additional analyzes such as positional cloning (Bouchard et al., 2011a). Technological advances have made the GWLS almost obsolete since the introduction of microarray-based, high-throughput SNP-genotyping methods, with the latter having drastically increased the ability to capture the existing variation in the genome of individuals (Bouchard and Malina, 2014). The so-called genome-wide association study (GWAS) is able to evaluate hundreds of thousands of SNPs rather than genomic loci, and individual data rather than familial data. Therefore, this approach is becoming increasingly popular in the search for polymorphisms that contribute to complex traits (Visscher et al., 2012).

In 2011, the first GWAS for the trainability of $\dot{V}O_{2max}$ was undertaken based on 324,611 SNPs, and interestingly, almost 50% of the variance in $\dot{V}O_{2max}$ response to an endurance training program was found to be predicted with a panel of 21 SNPs (Bouchard et al., 2011b). More recently, the GWAS approach was undertaken to identify variants associated with endurance athletic status (Ahmetov et al., 2015; Rankinen et al., 2016). In the first study, 1,140,419 SNPs were evaluated in 80 international-level Russian endurance athletes, in which six polymorphisms were more significantly associated with the endurance athletes (Ahmetov et al., 2015). In the second study, 195,000 and 700,000 SNPs were evaluated in two cohorts of elite endurance athletes (GENATHLETE and Japanese endurance runners, respectively), from which a panel of 45 promising SNPs was highlighted (Rankinen et al., 2016). Interestingly and also intriguing, seven additional cohorts of endurance athletes and controls were subsequently used for replication of the highlighted SNPs, through case-control association studies, and only one SNP (rs558129 at *GALNTL6* locus) showed the same direction of association in all replication cohorts (Rankinen et al., 2016).

An empirical evaluation of the early experience with GWAS suggests that non-replicating results and inconsistency in the magnitude of results are common features of this approach (Ioannidis, 2007). An expressive amount of information is generated by genome-wide approaches; however, some possibilities need to be considered. Like any other study of genetic association, the following situations are possible: (i) a significant association is found, and the result is replicated in other cohorts, which can indicate a true-positive result; (ii) a significant association is found, but replication studies do not confirm the association, which can indicate a false-positive result; or (iii) the replication study was not sensitive enough to detect previous associations, which can indicate a methodological flaw (Ioannidis, 2007). For all variants in which an initial association was observed (regardless of the method used), replication studies are of paramount importance. Consistent associations strengthen the evidence of the influence of the polymorphism on phenotype. However, even if the replication occurs in more than one study, it does not mean that the same association will be found in every cohort or population (Guilherme et al., 2014).

In the last version of the human gene map for performance and health-related fitness phenotypes, a total of 221 variants in the nuclear genome and 18 mtDNA markers have been shown to be associated with a relevant phenotype in at least one study (Bray et al., 2009). Regarding athlete genetics, a more recent literature review revealed that at least 155 genetic markers (i.e., nuclear genomic variants and mtDNA markers; 93 endurance-related, and 62 power/strength-related genetic markers) have been shown to be associated with a relevant phenotype in at least one study (Ahmetov et al., 2016). Of note, several of these variants were not replicated in additional studies and may be false-positive. Interestingly, 41 variants were identified very recently due to new GWAS approaches—indicating that GWASs represent a promising and productive method

(Ahmetov et al., 2016). However, it is noteworthy that GWAS captures most of the common SNPs found in the human genome, but do not cover all types of variants, such as the copy number variants. Moreover, the genomic architecture of complex traits also includes rare variants (Bouchard, 2015), for which we still have little information because they are hard to study. For instance, to uncover rare variants relevant to performance phenotypes, it may be necessary to sequence the genome of a large number of individuals on which the relevant trait has been measured (Bouchard, 2015). Although genetics has advanced in the recent years, there is still much to evolve to better understand the genetics of sports and exercise.

1.6 Research gaps and the athlome project consortium

Complex traits such as physical performance are influenced by both genetic and environmental factors, the aforementioned: nature and nurture influence. Although deliberate practice and environmental factors (i.e., nurture aspects) are undoubtedly both critical to performance, they do not in themselves produce outstanding physical performances such as highly elite athletes—some evidence has favored an important role of nature over nurture (Georgiades et al., 2017). In fact, several studies comparing variations at the DNA level have suggested that certain specific genes and variants are involved in athletic performance achievement (Ahmetov et al., 2016; Ahmetov and Fedotovskaya, 2015); however, the use of the traditional genetic association studies over the past decades in sports and exercise has had limited success to feature relevant gene variants with consistent replications in different cohorts (Tanaka et al., 2016). Genetic associations with performance phenotypes should be initially interpreted carefully and replicated in independent populations before being accepted as valid (Gibson, 2016). Of note, methodological characteristics between studies (e.g., sample size, ethnic background, and sample heterogeneity) have favored some inconsistency in the findings (Guilherme et al., 2014).

It is noteworthy that each variant can probably explain a very small proportion of the phenotypic variation, especially common variants such as SNPs (Bouchard, 2015). Therefore, very large sample sizes are needed to detect true associations in sports genetics. However, it is difficult to reconcile this premise with the scarce number of athletic champions worldwide for a given ethnicity and sport event, and thus, collaborations and data sharing between research centers worldwide have been recommended to circumvent this limitation and improve the sample size and quality (Eynon et al., 2011; Mattsson et al., 2016).

Since physical performance is the result of a combination of many different phenotypes, it will be interesting if future studies base genetic analyses on more detailed phenotyping (i.e., relevant variants and physiological data must be integrated) (Mattsson et al., 2016). Furthermore, to identify rare and other unknown variants further studies focused on genomic and mtDNA sequencing will serve as a reference panel for

future investigations. Nevertheless, the whole-genome sequencing of elite athletes alone will not be sufficient to meaningfully understand the mechanisms underlying the biology of physical performance. Indeed, this will require genomic and epigenomic information that can be potentially linked with transcriptomic and proteomic information (Tanaka et al., 2016). Ultimately, definitive proof that gene variants really favor relevant phenotypes would require a long-term prospective study. Taken together, all these approaches will help us to better understand the biology of physical performance.

In this regard, a recent consensus emerged among several research centers worldwide to collectively study the genotype and phenotype data currently available on elite athletes, in adaptation to exercise training and on exercise-related musculoskeletal injuries, named the Athlome Project Consortium (Pitsiladis et al., 2016). The main aim of the Athlome project is to characterize the genetics and biology of sports and exercise. To achieve this, several steps are set out as described in detail (Pitsiladis et al., 2016). Data generated from the Athlome Project will be made publically available for sharing of resources with the wider scientific community (http://athlomeconsortium.org/). The Athlome Project is a unique and highly ambitious attempt to uncover genetic variations and genomic process underlying sports and exercise biology in the coming years (Wang et al., 2016).

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CHAPTER TWO

Heritability estimates of physical performance-related phenotypes

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2.1 Introduction

Twins share many features such as face structure, height, and weight and children resemble their parents. Aren't such phenomena recognized by everyone? Monozygotic (MZ) twins are similar to clones and have exactly the same genome sequence. Thus, families share much matching genetic information. However, most families also share the same living environment such as living in the same house and consuming the same meals. Therefore, families share both very similar genetic information and environments. To determine whether genetic and environmental factors have a greater influence on phenotype, studies should compare MZ twin populations with nearly perfect agreement of genomic information at the same age and in the same living environment to a population of dizygotic (DZ) twins who share the same age and living environment and have some genetic similarity. Comparing these populations would determine whether similarities in these individuals are conferred by genes.

Fig. 2.1 shows the correlations of quantitative traits in twin pairs. A solid circle indicates an MZ twin pair. While an open circle indicates a DZ twin pair. Fig. 2.1A shows that the data for the pairs of MZ formed nearly a straight line with a gradient of 1 compared to the pairs of DZ. We assumed that inheritance affects phenotype. In contrast, Fig. 2.1B shows that the plots for MZ and DZ were similar, indicating the low impact of genetic involvement.

Reed et al. (1991) evaluated differences on in grip strength between twins. The grip strength of MZ showed an $r = 0.62$, while that of DZ was $r = 0.39$. Thus, MZ with greater genetic similarity exhibited a higher concordance rate with grip strength compared to DZ. The correlation coefficients for their twins showed that heredity affects physical fitness. However, this result alone does not clarify whether genetics has the stronger influence on physical fitness than environment. Accordingly, we calculate "heritability" as an indicator of genetic factors for comparison to "environmentality."

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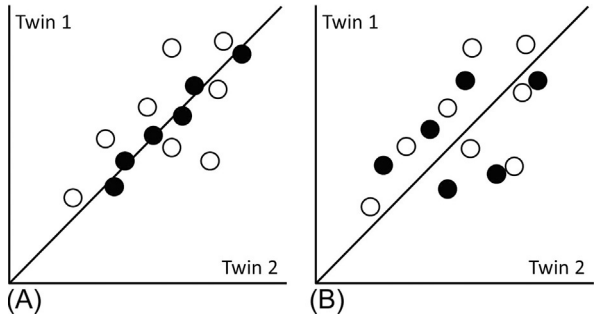

Fig. 2.1 Interpair comparison of quantitative trait for MZ and DZ twins. Solid circle indicates a pair of MZ. Open circle indicates a pair of DZ. (A) The correlation is higher in MZ than in DZ. It suggests inheritance affects phenotype. On the other hand, in (B), the plots for MZ and DZ were similar, indicating the low impact of genetic involvement.

In the fields of sports science and exercise, studies have been conducted to evaluate genetic factors affecting phenotypes related to sports performance and physical fitness (muscle strength, endurance capacity, etc.) (Bouchard et al., 1997). Regarding heritability as an indicator of the influence of genetic factors on phenotype, this paper focuses on muscular strength and endurance capacity, which are major factors affecting physical fitness. We also review the relationship between these factors and athletic status. Moreover, there are individual differences in trainability, and it has been reported that genetic factors are involved in these individual differences. In athletes, identifying the genetic factors affecting muscle strength and endurance capacity, which are the foundation of athletic performance, is considered to be a critical factor in competition selection and training methods suited to each individual. On the other hand, even among ordinary people, a high level of physical activity, exercise, and greater physical fitness are correlated with a reduced risk of disease and mortality. Thus, the importance of exercise and improving physical fitness has been recognized not only from the perspective of sporting competition, but also from that of health. Therefore, it is important to identify the genetic factors affecting physical fitness, physical activities, and exercise, as well as the extent of these effects and specific genes involved.

2.2 What is heritability estimate?

The anatomical structure and function of the human body (= phenotype) as well as the response to various external factors exhibit individual differences. In addition to environmental factors (living and regional environments, etc.) and socioeconomic factors (education, income, social support, etc.), genetic factors cause individual differences. Most human quantitative traits such as height (cm) and weight (kg) are determined partly by genetic variation (e.g., single-nucleotide polymorphism). However, these traits are polygenic and the involvement of a single-nucleotide polymorphisms on phenotype is small.

Therefore, in population genetics, rather than evaluating the effects of single gene, researchers measure the effects of an entire locus (heritability) using statistical models. The classical method for calculating the heritability estimate is derived from the difference in correlation within a pair of MZ and within a pair of DZ, or from the variance between twins. In the recent years, rather than the classical method, structural equation modeling (SEM) has become a widely used method of calculating the heritability estimate.

First, we explain the concept of heritability. A genetic factor refers to how genetic diversity can account for individual differences in human phenotypes. Heritability is a numerical representation of this factor and is the ratio $(\frac{\sigma_G^2}{\sigma_P^2})$ of the genetic variance (σ_G^2) to the total variance of the phenotype (σ_P^2), known as broad-sense heritability (H^2). We can express the relative ratio of genetic variance and environmental variance (σ_E^2) to phenotypic variance as well as compare the influence of genetic and environmental influences causing individual differences. However, if the heritability of muscle strength is 60% when a person grip strength 50 kg, it does not mean that 30 kg of this 60% is made by heredity and the remaining 20 kg is made in the environment. In other word, the heritability estimate merely explains the contribution ratio of genetic factors to the variation of phenotype in population. G is divided into an additive genetic effect (a) and a nonadditive genetic effect (d), and variance can be included as $\sigma_G^2 = \sigma_a^2 + \sigma_d^2$. Additive genetic effects are the additive effects of alleles at different loci. Nonadditive genetic effects include dominant effects and gene-gene interactions (epistasis effects). In addition, the ratio of σ_a^2 to σ_P^2 $(\frac{\sigma_a^2}{\sigma_P^2})$, or the condition under which the effect of σ_d^2 is neglected in genetic factors, is known as narrow-sense heritability and is expressed as h^2. Because nonadditive genetic effects (d) are determined by a combination of genes, inheritance of these effects is unlikely, and a greater h^2 means the more closely resembled parent and child are. Environmental factors can be classified into common environmental factors (c) shared among family and nonshared environmental factors (e) experienced by individuals $(\sigma_E^2 = \sigma_c^2 + \sigma_e^2)$. Nonshared environmental factor includes measurement errors.

Next, we explain the classical method of estimating heritability, which is very simple using sample variance (s^2). The variance of measured values in twin pairs is considered to indicate phenotypic variation. Heritability can be estimated by comparing the variance of MZ and DZ.

$$H^2 = \frac{{s_{DZ}}^2 - {s_{MZ}}^2}{{s_{DZ}}^2} \tag{2.1}$$

If ${s_{MZ}}^2 = {s_{DZ}}^2$, the numerator of Eq. (2.1) becomes 0 and the heritability estimate is 0. If ${s_{MZ}}^2 = 0$, the heritability estimate is 1, indicating that 100% inheritance affects the phenotype.

Next, we describe the method for estimating heritability based on the correlation coefficient for some phenotypes between twins. For both twin phenotypes, the influence of

nonadditive genetic effects (d) is ignored in the ACE model (A, additive genetic factors; C, common environmental factors; E, nonshared environmental factors). Genetic factors (a) and shared environments (c) between MZ twins are equal, and the only difference is the individual's own nonshared environment (e). Therefore, the degree of similarity of the phenotype between twins is explained by the genetic factors and shared environment. The correlation coefficient "r" of two variables equals their covariance divided by the product of their individual standard deviations. However, the r of two standardized variables can be expressed by using only their covariance: Thus, we can express $P = a + c + e$.

$$\begin{aligned} r_{MZ} &= Cov\left(P_{twin1}, P_{twin2}\right) \\ &= \Sigma(\mathrm{a}i + \mathrm{c}i + \mathrm{e}_{twin1}i)\,(\mathrm{a}i + \mathrm{c}i + \mathrm{e}_{twin2}i) \\ &= \Sigma\left(a_i^2 + c_i^2\right) + 2\Sigma\,\mathrm{a}ici + \Sigma(\mathrm{a}ie_{twin1}i + \mathrm{c}ie_{twin1}i + \mathrm{a}ie_{twin2}i + \mathrm{c}ie_{twin1}i + \mathrm{e}_{twin1}i\,\mathrm{e}_{twin2}i) \end{aligned}$$

a and c are independent of e, and the relationship between a and c is omitted here;

$$r_{MZ} = \Sigma a_i^2 + \Sigma c_i^2 \tag{2.2}$$

DZ share half of their genes inherited from the parents (the expected value of concordance is an average of 50%), and only the shared environment is equal between twins.

$$r_{DZ} = \frac{1}{2}\Sigma a_i^2 + \Sigma c_i^2 \tag{2.3}$$

By solving these two equations simultaneously, h^2 is calculated as follows (Falconer's formula) (Falconer 1960):

$$h^2 = 2\left(r_{MZ} - r_{DZ}\right) \tag{2.4}$$

For example, with respect to the correlation of grip strength between twins described by Reed et al. (1991) that introduced above, because $r_{MZ} = 0.618$ and $r_{DZ} = 0.385$, h^2 is 0.47. In addition, a (additive genetic factor) is 0.47, c ($= 2r_{DZ} - r_{MZ}$) is 0.15, and e ($= 1 - r_{MZ}$) is 0.38. The contribution ratio of the additive genetic factor and common environment in twins is shown in Fig. 2.2.

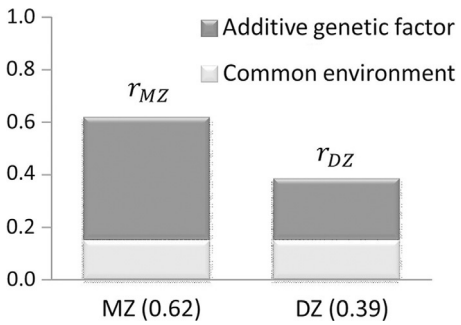

Fig. 2.2 The contribution ratio of additive genetic factor and common environment in twins.

If the correlation coefficient of the phenotype between MZ is large even after doubling the correlation coefficient of DZ ($2r_{DZ} < r_{MZ}$), the similarity of MZ is very high. There are two reasons for this: the effect of a special common environment that makes MZ similar or nonadditive genetic effects (d) are involved. If there is an effect of a specific common environment, the "equal environment assumption," where the degree of the common environment between pairs is the same both in MZ and DZ, has not been established. However, the equal environment assumption has been established (Koenig et al., 2010). Therefore, a specific common environment that makes MZ similar is unlikely. In addition, because common environment variance σ_c^2 can be calculated as $2r_{DZ} - r_{MZ}$, the effects of the common environment on the phenotype are negligible. In case of $2r_{DZ} < r_{MZ}$, the value for common environment ($2r_{DZ} - r_{MZ}$) becomes negative, and thus the model for the ACE is not unconformable. This suggests that the nonadditive genetic effect (d) affects the phenotype, the common environment is not considered in the genetic model (ADE model: A, additive genetic factors; D, nonadditive genetic factors; E, nonshared environment factors). The correlation coefficient of MZ is shown as

$$r_{MZ} = \Sigma a_i^2 + \Sigma d_i^2 \tag{2.5}$$

In contrast, in the case of DZ, the concordance rate of the gene combination as the dominant effect is 1/4 of MZ (combination of 1/2 from paternal inheritance and 1/2 from maternal inheritance). This is determined as follows:

$$r_{DZ} = \frac{1}{2}\Sigma a_i^2 + \frac{1}{4}\Sigma d_i^2 \tag{2.6}$$

Calculation of the heritability estimate using the correlation coefficient does not consider measurement errors. In the recent years, rather than the correlation coefficient, a method for calculating the heritability estimate using SEM has become widely used. SEM is a combination of path analysis and the factor analysis and estimates the covariance structure of actual data using a hypothetical model. Furthermore, in multivariate genetic analysis using SEM, it is possible to simultaneously calculate genetic factors and environmental factors common to multiple phenotypes, as well as genetic factors and environmental factors specifically involved in individual phenotypes. In addition, genetic and environmental factors in longitudinal data such as when determining the influence of development and aging can also be evaluated. Several different genetic models (ACE, ADE, AE, CE, E) are used in this calculation, and the model that best matches the observed pattern of similarity in MZ and DZ is chosen using a goodness-of-fit index. χ^2, Akaike's information criterion (AIC), Bayesian information criterion (BIC), etc. can be used as goodness-of-fit indices. Then, heritability is determined from the path coefficient in the selected genetic model.

In a recent metaanalysis of genetic factors for 17,804 traits (skeletal structure, metabolism, psychology and social interaction, etc.) in 14,558,903 individuals (twins/family)

from 2748 papers, the heritability was found to be approximately 50% for all traits (Polderman et al., 2015). The trait with the highest heritability showed a value of 71.2% in the ophthalmologic system, followed by 63.7% in the ears, nose, and throat, 60.4% in the skin, and 59.1% in the skeletal structure. Therefore, phenotypes are affected by genetic factors. Candidate genetic approaches and hypothetical-free approaches are being carried used to identify the specific genes related to these genetic factors. Particularly, the genome-wide association study (GWAS), which comprehensively analyze single-nucleotide polymorphisms in all chromosomal regions, has been widely conducted. However, the h^2 calculated by GWAS is problematic in that it is lower than the estimate, which is referred to as "missing heritability" (Eichler et al., 2010).

Models have been constructed in which genetic and environmental factors are independent ($\sigma_P^2 = \sigma_G^2 + \sigma_E^2$). However, a "gene-environment interaction" may occur between genetic and environmental factors. In the recent years, a method for detecting this interaction using SEM has been developed (Purcell, 2002), and studies using this model are ongoing.

2.3 Heritability estimates of muscle strength-related phenotypes

Skeletal muscle strength and power are, respectively, defined as the force produced by muscle contraction and product of force and velocity. They are important factors in health, activities of daily living, and athletic performance. Recently, a large longitudinal population study (139,691 participants from 17 countries) showed that grip strength is a stronger predictor of cardiovascular and all-cause mortality than systolic blood pressure (Leong et al., 2015). Moreover, a decrease in muscle strength (i.e., sarcopenia and dynapenia) is considered an important social issue because of the globally growing elderly populations in high-income societies. Muscle strength-related phenotypes have been reported to be influenced by genetic and many environmental factors, such as exercise habits, diet, and geographic factors. The heredity of muscle strength-related phenotypes was first examined in 1970 (Venerando and Milani-Comparetti, 1970). In the 1980s, researchers applied SEM to calculate heritability estimates. Nevertheless, the heritability estimates of muscle strength-related phenotypes (H^2-msp), defined as the proportion of genetic variation per total trait variation, varied widely from 0% to 98%. Therefore, we evaluated the H^2-msp by systematic review and meta-analysis derived from studies of twins and families (Zempo et al., 2017).

A systematic literature search was conducted using PubMed (through August 22, 2016) using the following keywords: (heredity or heritability) and ("muscle strength" or "muscle contraction" or endurance or athlete* or fitness or exercise) and (twin* or family). We searched English or Japanese language studies involving human subjects. Studies reporting the H^2-msp for healthy subjects in a sedentary state were included. We identified 1988 articles from the searches with filtering. After screening of titles

and abstracts, 84 articles were subjected to full-text screening, and an additional 34 studies were added by manual searching and based on the reference lists of the screened 84 articles. After reading the articles in detail, 94 articles were excluded: reviews, other measures of physical performance, lack of quantitatively comparable data, and duplicate reports of the same cohort. A total of 24 articles were selected for the metaanalysis, representing 58 strength-related phenotype measurements (Fig. 2.3). These measurements were classified into six phenotypic categories: 12 isometric grip strength, 16 other isometric strength, 9 isotonic strength, 9 isokinetic strength, 8 jumping ability, and 4 other power measurements. A total of 330,902 participants from 24 articles were included in the analysis. One of 24 articles included 316,832 participants (Silventoinen et al., 2008). The average age of the subjects was less than 20 years in 8 articles (33.3%), 20–39 years in 9 articles (37.5%), and over 40 years in 7 articles (29.2%). Examination of the heritability rate by muscle force demonstration style revealed a wide distribution of the heritage rate in both cases. Of the 38 measurements obtained using SEM models, the ADCE component was estimated for muscle strength-related phenotypes. In all, 21 studies used AE models and 12 used ACE models. Only two measurements showed a nonadditive genetic effect (D). The common environmental effect (C) ranged from 0 to 0.53.

Random-effects models were used to calculate the weighted mean heritability estimates. In all, 24 articles including 58 measurements were included in the meta-analysis.

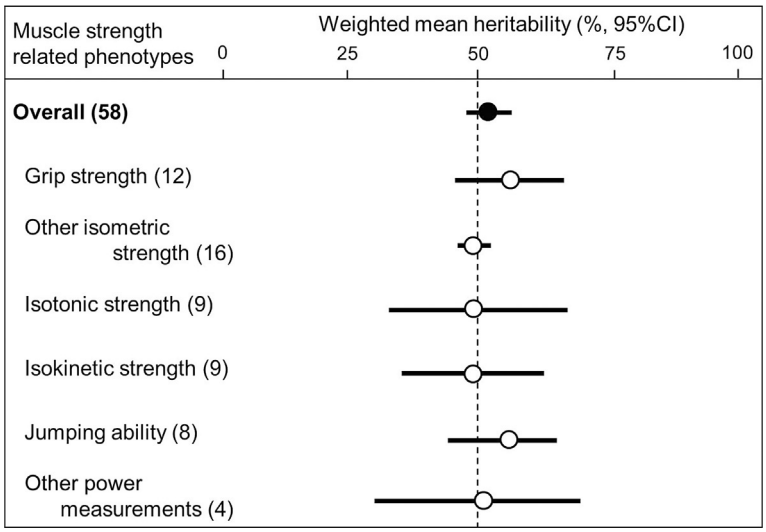

Fig. 2.3 Forest plot of a metaanalysis of the heritability estimates of the muscle strength-related phenotypes for 58 measurements in 24 articles. Summed values of additive (*a*) and nonadditive (*d*) genetic effect. *Solid circle*, the weighted mean heritability. *Line*, 95% confidence intervals; (number), number of measurement.

The weighted mean H^2-msp was 0.52 [95% confidence intervals (CIs): 0.48–0.56] (Fig. 2.3). This value agrees with that in a previous study reporting a heritability estimate of 0.49 for 17,804 human traits determined by metaanalysis (Polderman et al., 2015). In addition, the value is lower than the heritability estimates for anthropometric measures such as height and body mass index (0.65 − 0.93) (Maes et al., 1997; Schousboe et al., 2003; Silventoinen et al., 2003; Visscher et al., 2006), therefore, a unique environmental variation (including measurement error) may have a greater effect on muscle strength than on anthropometric measures. Subgroup analysis showed that the heritability estimates of isometric grip strength, other isometric strength, isotonic strength, isokinetic strength, jumping ability, and other power measurements were 0.56 (95% CI, 0.46–0.67), 0.49 (0.47–0.52), 0.49 (0.32–0.67), 0.49 (0.37–0.61), 0.55 (0.45–0.65), and 0.51 (0.31–0.70), respectively. These values did not significantly different from each other ($p = 0.486$). We found a considerable degree of heterogeneity ($I^2 = 91.0\%$, $p < 0.001$) in the weighted mean H^2-msp of all measurements and for isometric grip strength ($I^2 = 96.2\%$, $p < 0.001$), other isometric strength ($I^2 = 54.4\%$, $p = 0.005$), and isotonic measurements ($I^2 = 52.5\%$, $p = 0.032$), individually. To identify the factors affecting these heterogeneities, we conducted sensitivity analysis. With respect to age category, H^2-msp values were 0.60 (95% CI, 0.54–0.66), 0.50 (0.43–0.57), and 0.43 (0.31–0.54) for <20, 20–40 and >40 years old, respectively ($p < 0.05$). Meta-regression analysis also indicated that the H^2-msp decreased with age ($r = -0.43$, $p < 0.05$).

The H^2-msp of our meta-analysis showed high heterogeneity which decreased with age. In contrast, the value was constant among phenotypic categories, which were divided into isometric grip strength, other isometric strength, isotonic strength, isokinetic strength, jumping ability, and other power measurements. The present study showed that the H^2-msp was related to age. Indeed, sensitivity analysis showed that the H^2-msp was inversely correlated with age. Carmelli and Reed (2000) studied the changes in grip strength in one group of subjects over 10 years and examined the heritability in the baseline and that 10 years later. For the muscular strength heritability estimate of 63- and 73-year-old people 77 MZ and 75 DZ, a 35% heritability estimate was reported for the 63-year-old subjects, while this value decreased 22% in 73-year-old subjects. This suggests that the heritability of grip strength declines with age and the effects of environmental factors increases. Few studies have examined the heritability of muscular strength, by tracking the same group over a long period of time. In future studies, it is necessary to further examine the temporal and sequential changes in heritability with regard to individual differences in muscular strength.

The H^2-msp was higher in men [0.56 (95% CI, 0.50–0.62)] than in women [0.35 (0.07–0.64)], although there was no significant difference ($p = 0.115$). The H^2-msp values were not affected by bias of publication year, estimation method, or number of subjects according to the meta-regression analysis, nor by publication bias according to Egger's test ($p = 0.928$).

The muscle fiber type affects muscle strength. Particularly, fast-type fibers produce explosive contractions and high strength. Previous studies reported that over 45% of the total variance in the proportion of muscle fiber type is explained by genetic factors (Komi et al., 1977; Simoneau and Bouchard, 1995) and does not greatly change in response to resistance training (Staron et al., 1994; Kosek et al., 2006). Therefore, interindividual variations in muscle fiber type may contribute to H^2-msp.

A study focusing on 217 MZ and DZ aged between 66 and 75 years old was conducted to calculate the heritability of the four following phenotypes: fastest walking speed, isometric knee extension strength, leg extension power, and muscle cross-sectional area (Tiainen et al., 2007). The results indicate that contributing hereditary factors exist which are shared across all phenotypes. Another study examining the common hereditary factors of the muscle cross-sectional area of elbow flexion, isometric strength, and isotonic strength of 41 MZ and DZ aged 17–30 years old also identified hereditary factors that are shared across these areas (De Mars et al., 2007). Some genetic factors for specific phenotypes may involve certain genetic polymorphisms. However, it remains unclear to what extent the over 20 polymorphisms reported to be related to muscle strength (Bray et al., 2009; Roth et al., 2012; Perusse et al., 2013) explain the H^2-msp. The present metaanalysis encourages the use of a gene-based approach (e.g., genetics and/or epigenetics) to identify genetic factors contributing to interindividual variations in muscle strength.

In conclusion, our results indicate that the influence of genetic and environmental factors on muscle strength-related phenotypes is comparable. Moreover, the effects of environmental factors increased with age. However, other environmental factors were not extracted from the studies included for metaanalysis. Because the gene-environment interaction may influence heritability, environmental factors such as daily physical activity and food intake should be examined in further studies.

2.4 Heritability estimates of endurance-related phenotypes

Endurance capacity is defined as the capacity to sustain a physical activity for the longest time possible. Endurance capacity is associated with both the performance in endurance sports such as marathons and health. Indeed, many epidemiological studies have shown that high cardiorespiratory endurance is associated with a reduced risk of type 2 diabetes, hypertension, cardiovascular disease, and all-cause mortality (Kodama et al., 2009; Kokkinos, 2014; Zaccardi et al., 2015). Therefore, it is important to clarify the factors affecting endurance capacity.

Maximal oxygen uptake (VO_{2max}) is often used as an index of endurance capacity. An early twin study reported that the heritability of VO_{2max} accounted for approximately

90% of the total variance (Klissouras, 1971). In contrast, another twin study suggested that no genetic component affected VO_{2max} (Komi and Karlsson, 1979). After these early studies, many twin and family studies have been conducted to estimate the heritability of VO_{2max}. However, the estimated heritabilities showed wide variation across studies.

Schutte et al. (2016) reported metaanalysis from studies of twins to obtain a robust estimate for the heritability of VO_{2max} in children to young adults (10–30 years old). Their meta-analysis showed that the heritability estimates of VO_{2max} expressed in absolute values and adjusted for body weight were 0.59 and 0.72, respectively. Each estimate was calculated using data from five and four twin studies, respectively. They concluded that innate factors determine more than half of the individual differences in the VO_{2max} from childhood to young adulthood. In contrast, the effects of differences in subject characteristics (i.e., age, sex, and exercise habits), exercise mode of the endurance test (i.e., cycling or running), and estimation method for heritability (i.e., correlation coefficient or SEM) on the heritability estimates of VO_{2max} remained unclear. Furthermore, although several studies reported heritability estimates for endurance-related phenotypes other than VO_{2max}, such as endurance test performance and exercise economy, etc., the extent to which heritability differs between each phenotype remained unclear. Therefore, to clarify the effects of these potential confounding factors on the heritability estimates of VO_{2max} and the differences in the extent of genetic components between each endurance-related phenotype, we performed meta-analysis (Miyamoto-Mikami et al., 2018) of the heritability estimates of endurance-related phenotypes based on the data collected in our systematic review which described in "Heritability estimates of muscle strength-related phenotypes" section. We identified 15 articles reporting heritability estimates for endurance-related phenotypes. Among the 15 articles, 10 reported heritability estimates for VO_{2max} or VO_{2peak}, while two articles reported heritability estimates for endurance test performance. Additionally, heritability estimates for submaximal phenotypes such as VO_2 at submaximal absolute intensities (50, 75, 100, 125, and 150 W), VO_2 at submaximal relative intensities [heart rate = 150 beats/min, respiratory exchange ratio (RER) = 0.95, ventilator threshold (VT), 60% and 80% VO_{2max}], and power output at submaximal relative intensity (heart rate = 150 beats/min) were reported in five articles. Our meta-analysis showed that the weighted mean heritability of all VO_{2max} measurements was 0.59 (95% CI, 0.52–0.66) (Fig. 2.4). Using adjusted methods to evaluate VO_{2max} showed that the weighted means of the heritability of absolute values and those adjusted for body weight and fat-free mass were 0.68 (95% CI, 0.59–0.77), 0.56 (0.47–0.65), and 0.44 (0.13–0.75), respectively (Fig. 2.4). The weighted mean heritabilities of VO_{2max} were significantly different when the different adjustment methods were used, suggesting that the adjustment method used influences the heritabilities of VO_{2max}. However, heterogeneity was observed in each heritability estimate. To examine the effects of potential confounding factors on the heritability estimates of VO_{2max}, we conducted sensitivity analysis. This analysis revealed that the heritability estimation method

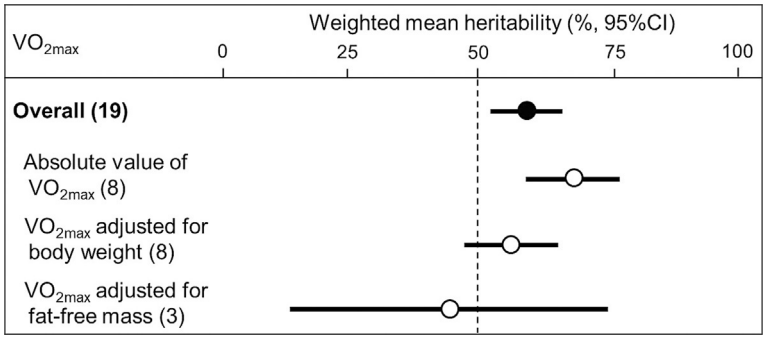

Fig. 2.4 Forest plot of a metaanalysis of the heritability estimates of VO_{2max} for 19 measurements in 15 articles. Summed values of additive (*a*) and nonadditive (*d*) genetic effect. *Solid circle,* the weighted mean heritability. *Line*, 95% confidence intervals; (number), number of measurement.

(SEM and others), study type (twin, family, and both), and exercise mode (cycle ergometer and treadmill) did not significantly influence the heritability estimates of VO_{2max} ($P \geq .174$). In contrast, the heritability estimates of VO_{2max} were significantly influenced by sex. Specifically, the weighted mean heritability of VO_{2max} was significantly higher in studies comprising only male subjects [0.69 (95% CI, 0.55–0.83)] compared to those comprising both male and female subjects [0.49 (0.42–0.56)]. When sex was considered, the heterogeneities of the heritability estimates of VO_{2max} among studies were no longer significant. Although no studies have examined only female subjects and calculated the heritability estimate of VO_{2max} adjusted by body weight, our results suggest that sex influences the heritability estimates of VO_{2max}. As described above, for muscle strength-related phenotypes, heritability was higher in males than in females. This is consistent with the VO_{2max}, although muscle strength-related phenotypes did not significantly differ. In contrast, meta-analysis of the heritabilities of all human traits based on twin studies conducted in the past 50 years reported that heritability estimates of all human traits were similar in both sexes (i.e., 0.465 in males and 0.472 in females) (Polderman et al., 2015). Therefore, the effects of sex on heritability estimates may differ between phenotypes; particularly, for physical performance-related phenotypes, males may be more strongly influenced by genetic factors.

In various submaximal phenotypes, VO_2 at absolute intensities reflects exercise economy, which partly explains the variability in endurance performance among subjects with similar VO_{2max} values (Bassett Jr. and Howley, 1997). Additionally, VT is associated with sustainable aerobic work capacity and aerobic fitness (Gaskill et al., 2001) and an RER of 0.95 reflects the anaerobic contribution to energy generation during exercise (Fagard et al., 1991). Therefore, these submaximal phenotypes are important components in endurance capacity. Our metaanalysis showed that the weighted mean heritability estimate of the submaximal phenotype was 0.49 (95% CI, 0.33–0.65), and that substantial heterogeneity was observed among studies. Although submaximal phenotypes included

various intensity phenotypes, there was no significant relationship between exercise intensity (%VO_{2max}) and the heritability estimates. Further extensive studies are necessary to clarify the factors that affect the heterogeneity.

The weighted mean heritability estimate of endurance test performance was 0.53 (95% CI, 0.27–0.78). There are only two published studies on heritability estimates of endurance test performance. Bouchard et al. (1986) reported that the heritability of total work output during 90 min of maximal exercise was 0.66. Another study by Ortega-Alonso et al. (2009) reported that the heritability of walking distance in a 6-min walking endurance test was 0.40. Participants in the latter study were elderly women with an average age of 68 years. Our metaanalysis did not identify a significant relationship between age and the heritability estimate of endurance-related phenotypes in contrast to muscle strength-related phenotypes. However, the age of the participants in the 15 studies selected for this metaanalysis was skewed to a relatively young age. Therefore, we cannot exclude the possibility that age affects the heritability of endurance-related phenotypes. In addition, because it is known that physical activity levels influence endurance capacity, we attempted to examine the effects of physical activity levels on heritability of endurance-related phenotypes. However, because of the lack of available information in several studies and because of differences in the methods of the assessment of physical activity levels among studies, we could not assess the effect of physical activity levels on heritability estimates of endurance-related phenotypes. Our systematic review and meta-analysis suggest the comparable importance of both genetic and environmental factors in endurance-related phenotypes.

The trainability of endurance capacity also seems to be influenced by genetic factors. Bouchard's group investigated heritability estimates of response of VO_{2max} and of submaximal phenotypes to 20 weeks of endurance training. Bouchard et al. reported that heritability of VO_{2max} response to 20 week endurance training program was 0.47 based on 481 individuals from 98 two-generation families (Bouchard et al., 1999). In addition, it has been reported that heritability estimates of response of submaximal phenotypes to 20-week endurance training were 0.22–0.57 (Gaskill et al., 2001; Perusse et al., 2001). Because few studies investigated the heritability of trainability of endurance capacity, further studies are needed to support the conclusion.

2.5 Heritability estimates of athletic performance

To our knowledge, in identical twin athletes, it has not been shown that one has been successful in sprint/power sports events and another one has been successful in the endurance sports event. A person with continued suitable training for a certain sports event will be better than someone without training. However, to be successful as an elite athlete like an Olympian, one should have both optimal genes and suitable training. Thus, it is not necessarily correct that "Hard work always pays off" in sports.

Athletic performance is a complex multifactorial phenomenon determined by numerous intrinsic factors, including genetics and sex, extrinsic factors, such as training and nutrition, and the interplay between these factors. Thus, it would be reasonable to consider that both genetic and environmental factors play an important role in determining athletic performance.

Several studies have reported significant heritability estimates for phenotypes relevant to athletic performance. Early twin and family studies suggested that 30%–80% of the variance in athletic performance was explained by genetic factors (Bouchard and Rankinen, 2001). To date, one large cohort study reported heritability estimates of athletic performance (De Moor et al., 2007). The authors recruited 4488 adult female twins (793 MZ, 1000 DZ, and single twins; mean age 51.9 ± 12.8 years, range 20–83 years) from the UK Adult Twin Registry. The athletic status was examined by asking the twins whether they had ever competed in sports and their highest level obtained. This cohort included sports such as running, swimming, tennis, gymnastics, badminton, dancing, and aerobics. Of the 4488 participants, 311 female athletes had competed at the county or national level ($n = 311$, 6.9%) and were considered elite athletes. In addition, 1679 (37.4%) had participated in sports at the school, club, or university level. The remaining 2498 (55.7%) had never participated in any organized sport. The authors reported a polychoric twin correlation in MZ and DZ of 0.66 (95% CI: 0.59−0.71) and 0.32 (0.24−0.40), respectively. Based on the pattern of MZ-DZ correlations (i.e., the additive genetic and nonshared environmental effects model), 65.5% variance in athletic status was explained by genetic factors and 34.5% variance in athletic status was explained by environmental factors (Fig. 2.5). As described above, heritability estimates are affected by factors that include age and sex (Zempo et al., 2017; Miyamoto-Mikami et al., 2018). The heritability estimates for athletic performance-related phenotypes, such as muscle strength, are higher in males than in females and decrease with age. For elite athletes,

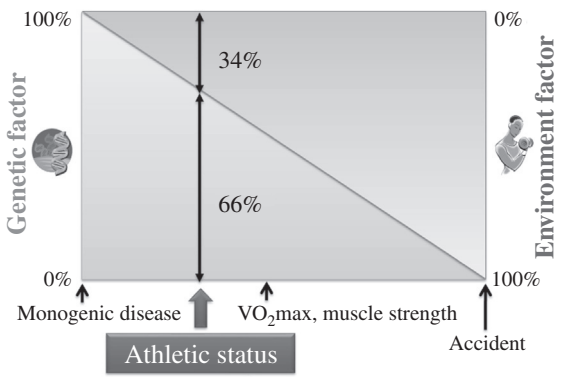

Fig. 2.5 Heritability estimates of athlete status. *(Based on the data of De Moor, M.H., Spector, T.D., Cherkas, L.F., Falchi, M., Hottenga, J.J., Boomsma, D.I., De Geus, E.J., 2007. Genome-wide linkage scan for athlete status in 700 British female DZ twin pairs. Twin Res. Hum. Genet. 10, 812–820.)*

such as Olympians (especially males), the heritability estimates of athletic performance may be higher than the 65.5% reported by De Moor et al. (2007), because the subjects of this study were middle-aged women. Thus, it is evident that genetic factors play an important role in determining athletic performance.

2.6 Conclusion

Individual differences in anatomical structure and functions can be found in human. One of the factors contributing to these individual differences is the genetic factor, and much research has been done on twins and families to study heritability. As mentioned above, although many studies link physical fitness to genetic factors, the range of the calculated heritability is very large. In the future, further research needs to be conducted on the factors (age, sex, race, environment, and evaluation method) that lead to this heterogeneity in heritability. In addition, apart from genetic abilities, it is necessary for athletes to increase an appropriate amount of proper training and the amount of their daily physical activities to gain high physical fitness. If we look at the "implementation" and "continuation" of an appropriate amount of training as an "exercise behavior," some genetic factors could be involved in this "exercise behavior" too. So far, there has been no research on the genetic factors of the implementation and continuation of training in athletes. However, there are studies that aim to clarify the genetic factors that cause individual differences in the "amount of daily physical activities," "exercise participation," and "continuation of training" in the general population. Research on how far genetic factors influence physical activities and exercise behavior has been conducted ever since the 1980s (Heller et al., 1988). These physical activities and exercise behavior are evaluated as the total amount of daily physical activities, exercise participation during leisure time and the amount of physical activities during leisure time. Although the heritabilities show a large range, they are reported to be 0%–85% (Murakami et al., 2016). Stubbe et al. (2006) conducted a study on the exercise participation of 37,051 pairs of MZ and DZ from 7 countries and calculated the heritability. Questionnaires were used to study the exercise participation during leisure time, and subjects who carried out exercises above 4 metabolic equivalents (METs) for more than an hour every week were categorized into the exercise group, while the others were categorized into the nonexercise group. According to the results, although the heritability differed in different countries, the male subjects showed a heritability of 27%–67%, while the female subjects showed a heritability of 48%–71%. The heritability of physical activities and exercise behavior varies in different studies and this could be due to the different evaluation methods of physical activities and exercises, and threshold values of exercise participation, adopted in different research, in addition to the different contribution levels of environmental factors, which are based on the cultural and social differences of the subject populations. On the other hand, research on mechanisms, which are the genetic factors of physical

activities and exercise behavior, has also been conducted, and it has been suggested that the desire to exercise could be a genetic factor that contributes to dispersion in physical activities and exercise behavior. Furthermore, much research on the genetic factors of sedentary activity and inactivity have begun to be conducted in the recent years. The clarification of these heritabilities and their mechanisms is expected to lead to methods to approach transformation in physical activities and exercise behavior and promotion of health issues. At the same time, by exploring and identifying genetic polymorphism, which forms the base of genetic factors, biological understanding about exercise abilities, physical activities, and exercise behavior may be deepened.

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CHAPTER THREE

Genes and power athlete status

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3.1 Introduction

Genetic factors have a great influence over phenotype components such as strength, power, flexibility, neuromuscular coordination, and psychological traits which are crucial for power/strength athletes. In the genome-wide linkage scan for athlete status, the heritability of athlete status was estimated at 66% (De Moor et al., 2007). However, the detailed rates differ depending on traits: for example, the heritability of muscle strength has been shown to range from approximately 30% to 80% in various phenotypes such as isometric knee strength, handgrip strength, and elbow flexion (Hughes et al., 2011). A literature review revealed at least 69 markers which are associated with power (sprinters, strength, and speed-strength) athlete status (Table 3.1), described below.

3.1.1 *ACE* rs4646994 D allele

ACE gene encodes an angiotensin I converting enzyme (ACE) involved in catalyzing the conversion of angiotensin I into a physiologically active peptide angiotensin II. Angiotensin II is a potent vasopressor and aldosterone-stimulating peptide that controls blood pressure and fluid-electrolyte balance. This enzyme is the most important component of the renin-angiotensin system (RAS).

The I/D polymorphism of the *ACE* gene (location: 17q23.3) denotes a substantial individual variation in RAS activity with the D allele being associated with higher ACE activity. Reported effects of *ACE* (I/D) polymorphism vary across studies and populations. Several studies have shown the D allele to be associated with greater strength and muscle volumes and an increased percentage of fast-twitch muscle fibers (Zhang et al., 2003; Charbonneau et al., 2008). In addition, the D allele and/or DD genotype was shown to be over-represented in British (Myerson et al., 1999), Russian (Nazarov et al., 2001), European and Commonwealth Caucasian swimmers (<400 m)

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Table 3.1 Genetic markers for power/strength athlete status

Gene	Name	Locus	rs number	Alternative polymophism symbols	Power/ strength-related marker	Studies with positive results		Studies with negative or controversial results	
						Number of studies	Total number of studied athletes from power/strength groups	Number of studies	Total number of studied athletes from power/strength groups
ACE	Angiotensin I converting enzyme	17q23.3	rs4646994	Alu I/D	D	7	385	8	779
ACVR1B	Activin A type IB receptor	12q13.13	rs2854464	A/G	A	1	399	1	180
ACTN3	Alpha-actinin-3	11q13.1	rs1815739	C/T, R577X, Arg577Ter	C (R, Arg577)	11	1424	6	659
ADRB2	Adrenoceptor beta 2	5q31-q32	rs1042713	G/A, Gly16Arg	G (Gly16)	1	100	–	–
	Adrenoceptor beta 2	5q31-q32	rs1042714	C/G, Gln27Glu	G (27Glu)	1	100	–	–
AGT	Angiotensinogen	1q42.2	rs699	T/C, Met235Thr	C (235Thr)	2	163	–	–
AGTR2	Angiotensin II receptor type 2	Xq22-q23	rs11091046	A/C	A	2	923	2	451
AMPD1	Adenosine monophosphate deaminase 1	1p13	rs17602729	C/T, Gln12X	C (Gln12)	3	510	–	–
ARHGEF28	Rho guanine nucleotide exchange factor 28	5q13.2	rs17664695	A/G	G	1	380	–	–
CACNG1	Calcium voltage-gated channel auxiliary subunit gamma 1	17q24	rs1799938	G/A, Gly196Ser	A (196Ser)	1	380	–	–
CALCR	Calcitonin receptor	7q21.3	rs17734766	A/G	G	1	380	–	–
CKM	Creatine kinase, M-type	19q13.32	rs8111989	A/G, *Nco*I	G	5	698	–	–
CLSTN2	Calsyntenin 2	3q23	rs2194938	A/C	C	1	319	–	–
CNDP1	Carnosine dipeptidase 1	18q22.3	rs2887	G/A	A	1	415	–	–
			rs2346061	A/C	C	1	415	–	–
CNDP2	Carnosine dipeptidase 2	18q22.3	rs3764509	C/G	G	1	415	–	–
CNTFR	Ciliary neurotrophic factor receptor	9p13.3	rs41274853	C/T	T	1	211	–	–
COTL1	Coactosin like F-actin binding protein 1	16q24.1	rs7458	C/T	T	1	380	–	–

CREM	cAMP responsive element modulator	10p11.21	rs1531550	G/A	A	1	257	–	–
DMD	Dystrophin	Xp21.2	rs939787	C/T	T	1	188	–	–
EPAS1 (HIF2A)	Endothelial PAS domain protein 1	2p21-p16	rs1867785	A/G	G	1	338	–	–
			rs11689011	C/T	C	1	338	–	–
FOCAD	Focadhesin	9p21	rs17759424	A/C	C	1	319	–	–
GABRR1	Gamma-aminobutyric acid type A receptor rho1 subunit	6q15	rs282114	A/G	A	1	380	–	–
GALNT13	Polypeptide *N*-acetylgalactosaminyltransferase 13	2q24.1	rs10196189	A/G	G	1	257	–	–
GPC5	Glypican 5	13q31.3	rs852918	G/T	T	1	380	–	–
HIF1A	Hypoxia inducible factor 1 alpha subunit	14q21-q24	rs11549465	C/T, Pro582Ser	T (582Ser)	4	550	1	81
HSD17B14	Hydroxysteroid 17-beta dehydrogenase 14	19q13.33	rs7247312	A/G	G	1	380	–	–
IGF1	Insulin-like growth factor 1	12q23.2	rs35767	C/T, C-1245T	T	1	87	–	–
IGF1R	Insulin-like growth factor 1 receptor	15q26.3	rs1464430	A/C	C	1	82	–	–
IGF2	Insulin-like growth factor 2	11p15.5	rs680	A/G	G	1	170	–	–
IL1RN	Interleukin-1 receptor antagonist	2q14.2	rs2234663	Intron 2 VNTR 86-bp, IL1RN*1-IL1RN*5	IL1RN*2	1	205	–	–
IL6	Interleukin 6	7p21	rs1800795	C/G, -174 C/G	G	2	211	1	81
IP6K3	Inositol hexakisphosphate kinase 3	6p21.31	rs6942022	C/T	C	1	380	–	–
MCT1 (SLC16A1)	Monocarboxylate (lactate/pyruvate) transporter 1/solute carrier family 16 member 1	1p12	rs1049434	A/T, A1470T Glu490Asp	T (490Asp)	1	100	3	397
MED4	Mediator complex subunit 4	13q14.2	rs7337521	G/T	T	1	380	–	–
MPRIP	Myosin phosphatase Rho interacting protein	17p11.2	rs6502557	A/G	A	1	380	–	–

Continued

Table 3.1 Genetic markers for power/strength athlete status—cont'd

Gene	Name	Locus	rs number	Alternative polymophism symbols	Power/ strength-related marker	Studies with positive results		Studies with negative or controversial results	
						Number of studies	Total number of studied athletes from power/strength groups	Number of studies	Total number of studied athletes from power/strength groups
MtDNA loci	Mitochondrial DNA	MtDNA	Haplogroups constructed from several MtDNA polymorphisms or single polymorphisms	Favorable:	F	1	60	–	–
					m.204C, m.151T, m.15314A,	1	85	–	–
					Non-L/U6	1	119	–	–
				Unfavorable:	m.16278T, m.5601T, m.4833G, m.5108C, m.7600A, m.9377G, m.13563G, m.14200C, m.14569A	1	85	–	–
MTHFR	Methylenetetrahydrofolate reductase	1p36.3	rs1801131	A/C, A1298C	C	2	933	–	–
MTR	5-Methyltetrahydrofolate-homocysteine methyltransferase	1q43	rs1805087	A/G, A2756G	G	1	77	–	–
MTRR	5-Methyltetrahydrofolate-homocysteine methyltransferase reductase	5p15.31	rs1801394	A/G, A66G	G	1	77	–	–
NOS3	Nitric oxide synthase 3	7q36	rs2070744	T/C, -786 T/C	T	4	278	–	–
			rs1799983	G/T, Glu298Asp	G (Glu298)	1	29	–	–
NRG1	Neuregulin 1	8p12	rs17721043	A/G	A	1	380	–	–
PPARA	Peroxisome proliferator activated receptor alpha	22q13.31	rs4253778	G/C	C	2	227	1	81
PPARG	Peroxisome proliferator activated receptor gamma	3p25.2	rs1801282	C/G, Pro12Ala	G (12Ala)	3	552	–	–

PPARGC1A	Peroxisome proliferative activated receptor gamma coactivator 1 alpha	4p15.2	rs8192678	G/A, Gly482Ser	A (482Ser)	1	161	1	513
PPARGC1B	Peroxisome proliferative activated receptor gamma coactivator 1 beta	5q32	rs10060424	C/T	C	1	380	–	–
RC3H1 (ROQUIN)	Ring finger and CCCH-type domains 1	1q25.1	rs767053	A/G	G	1	380	–	–
SOD2	Superoxide dismutase 2	6q25.3	rs4880	C/T, Ala16Val	C (Ala16)	2	598	–	–
SUCLA2	Succinate-CoA ligase ADP-forming beta subunit	13q14.2	rs10397	A/C	A	1	380	–	–
TPK1	Thiamin pyrophosphokinase 1	7q34-q35	rs10275875	C/T	C	1	319	–	–
TRHR	Thyrotropin-releasing hormone receptor	8q23.1	rs7832552	C/T	T	2	151	–	–
UCP2	Uncoupling protein 2	11q13.4	rs660339	C/T, Ala55Val	C (Ala55)	1	29	–	–
WAPL	WAPL cohesin release factor	10q23.2	rs4934207	C/T	C	1	380	–	–
ZNF423	Zinc finger protein 423	16q12.1	rs11865138	C/T	C	1	380	–	–

(Woods et al., 2001), Greek sprinters (Papadimitriou et al., 2009), Portuguese (Costa et al., 2009), Spanish strength/power athletes (Boraita et al., 2010), and Caucasian short-and-middle-distance swimmers (Wang et al., 2013). Contrary to these findings, other report has shown that Korean top-level power-oriented athletes had a markedly diminished frequency of the D allele than national-level power-oriented athletes or controls (Kim et al., 2010). The same finding was reported by studying power-oriented Lithuanian and Russian athletes and controls (Ginevičienė et al., 2011; Gineviciene et al., 2016) as well as in Iranian population (Shahmoradi et al., 2014), respectively. In addition, Wang et al. (2013) reported that East Asian short-distance swimmers have a higher prevalence of the *ACE* I allele in comparison with controls. Furthermore, several studies of power/sprint athletes have demonstrated no association between the *ACE* I/D polymorphism and power athlete status (Amir et al., 2007; Scott et al., 2010; Sessa et al., 2011).

3.1.2 *ACVR1B* rs2854464 A allele

The *ACVR1B* gene encodes an activin A type IB receptor (location: 12q13.13). Activins are dimeric growth and differentiation factors which belong to the transforming growth factor-beta (TGF-beta) superfamily of structurally related signaling proteins. Activins signal through a heteromeric complex of receptor serine kinases which include at least two type I and two type II receptors. This protein is a type I receptor which is essential for signaling. The *ACVR1B* rs2854464 A allele has previously been associated with increased muscle strength in healthy, nonathletic individuals (Windelinckx et al., 2011). In a relatively large cohort of athletes from Europe and South America, it has been shown that the *ACVR1B* rs2854464 A allele is associated with sprint/power athlete status in Caucasians but not in Brazilian athletes (Voisin et al., 2016).

3.1.3 *ACTN3* rs1815739 C allele (Arg577)

The *ACTN3* gene encodes the protein alpha-actinin-3, a sarcomeric protein that is expressed in fast, type II fibers, where it plays an important role in the generation of explosive and powerful muscle contractions.

A common genetic variation in the *ACTN3* gene (location: 11q13.1) that results in the replacement of an arginine (Arg or R) with a stop codon at amino acid 577 (C-to-T transition in exon 16; rs1815739; R577X) had been identified. The 577X allele contains a sequence change that completely prevents the production of functional α-actinin-3 protein. Several case-control studies reported that *ACTN3* RR genotype is over-represented or *ACTN3* XX genotype is under-represented in strength/sprint athletes in comparison with controls. Yang et al. (2003) for the first time have shown that the frequency of the *ACTN3* XX genotype was reduced in Australian power athletes compared to controls, whereas none of the Olympians or female power athletes had an XX genotype. These findings have been supported by the independent replications

in case-control studies of elite Finnish sprint athletes (Niemi and Majamaa, 2005), elite Greek track and field athletes (Papadimitriou et al., 2008), elite-level strength athletes from across the United States (Roth et al., 2008), Russian power-oriented athletes (Druzhevskaya et al., 2008), Italian artistic gymnasts (Massidda et al., 2009), Taiwanese sprint swimmers (Chiu et al., 2011), Israeli sprinters (Eynon et al., 2009), Russian short-distance speed skaters (Ahmetov et al., 2011), Polish power-oriented athletes (Cięszczyk et al., 2011), and Japanese sprint/power athletes (Mikami et al., 2014). It should be noted that several studies reported no association between the *ACTN3* R577X polymorphism and power athlete status (Yang et al., 2007; Scott et al., 2010; Ginevičienė et al., 2011; Sessa et al., 2011; Gineviciene et al., 2016).

In addition, Vincent et al. (2007) have shown that the percentage of the cross-sectional area and the number of type IIx (fast-twitch glycolytic) fibers was greater in the RR than the XX genotype group of young healthy men. This association was replicated in a second study, where the *ACTN3* R577X polymorphism was shown to be associated with muscle fiber composition in a group of physically active men and sub-elite speed skaters, indicating that *ACTN3* XX genotype carriers exhibit a higher proportion of slow-twitch muscle fibers (Ahmetov et al., 2011; Ahmetov et al., 2012). It was also shown that the *ACTN3* R allele was associated with high levels of testosterone in both male and female athletes (Ahmetov et al., 2014a), and this may explain, in part, the association between the *ACTN3* RR genotype, skeletal muscle hypertrophy, and power athlete status.

3.1.4 *ADRB2* rs1042713 G allele (Gly16) and rs1042714 G allele (27Glu)

The β-2 adrenergic receptor (encoded by *ADRB2*; location: 5q31-q32) is a member of the G protein-coupled receptor superfamily, expressed in many cell types throughout the body and plays a pivotal role in the regulation of the cardiac, pulmonary, vascular, endocrine, and central nervous system.

The Gly16Arg single-nucleotide polymorphism (SNP) (rs1042713 G/A) of the *ADRB2* gene and its association with several phenotypes has been described. Specifically, the 16Arg allele was associated with lower receptor density and resting cardiac output (Snyder et al., 2006). Sawczuk et al. found that the Gly16 allele and the 27Glu variant of the Gln27Glu (rs1042714 C/G) polymorphism were over-represented in Polish strength/power athletes compared with controls (Sawczuk et al., 2013).

3.1.5 *AGT* rs699 C allele (235Thr)

The angiotensinogen (AGT), serum α-globulin formed by the liver, is an essential component of the RAS. The AGT is cleaved by the renin to form biologically inactive angiotensin I, the precursor of active angiotensin II that regulates vascular resistance and sodium homeostasis, and thus determining blood pressure.

The AGT is encoded by *AGT* gene (location: 1q42.2). Met235Thr polymorphism of the *AGT* gene leads to the substitution of threonine to methionine at position 235 (rs699 T/C). The *AGT* Met235Thr polymorphism was shown to be associated with left-ventricular mass index increase in a study of young healthy individuals after 17 weeks of exercise training (50%–80% VO_{2max}) (Lucia et al., 2006). Individuals with the *AGT* Thr/Thr genotype had significantly greater left-ventricular mass index than those with the Met/Met or Met/Thr genotype. Gomez-Gallego et al. compared the genotype and allele frequencies for the *AGT* Met235Thr variation of Caucasian athletes (world-class endurance athletes, power athletes, and nonathletic controls). Results revealed a higher percentage of Thr/Thr genotype carriers among power athletes than either in controls or an endurance group (Gomez-Gallego et al., 2009). These findings were replicated in a study of Polish power athletes (Zarębska et al., 2013). Therefore, it is assumed that 235Thr allele of the *AGT* Met235Thr polymorphism might favor power sports performance and this could be attributed to the higher activity of angiotensin II that acts as a growth factor in skeletal muscle.

3.1.6 *AGTR2* rs11091046 A allele

The *AGTR2* gene (location: Xq23) encodes the angiotensin II receptor type 2 that is an integrative part of the RAS, which plays a crucial role in the control of circulatory system and blood pressure. The RAS also controls skeletal muscle function by regulation of local perfusion, which can modulate metabolic activity. The main effector of the RAS is angiotensin II that acts via two major receptors: AGTR1 (that acts as a vasoconstrictor) and AGTR2 (with vasodilator function) (Carey, 2016).

The rs11091046 A allele was listed as a marker for power traits in different populations (Guilherme et al., 2014; Ahmetov and Fedotovskaya, 2015). By studying athletes from Russia and Poland, Mustafina et al. found that the frequency of the *AGTR2* A allele was significantly over-represented in female power athletes in comparison to control subjects. However, in the same study, the opposite trend has been observed with regard to C allele being over-represented in the group of male strength athletes (Mustafina et al., 2014). The latest report indicated that Brazilian female power athletes had also a higher frequency of the A allele. Furthermore, men sprinters with the A allele showed significantly faster personal best times for the 100 m than those with the C allele (Guilherme et al., 2018). On the other hand, the study of Japanese, Russian and Polish track and field sprint/power athletes showed a significantly higher frequency of the C allele in athletes than in controls. However, with regard to respective cohorts, C allele frequency was higher in Japanese male athletes than in controls, but not in Russian/Polish male athletes. In women, no significant results were obtained (Yvert et al., 2018).

3.1.7 *AMPD1* rs17602729 C allele (Gln12)

Adenosine monophosphate deaminase (AMPD) is an important regulator of muscle energy metabolism: by converting adenosine monophosphate (AMP) into inosine monophosphate (IMP) with liberation of ammonia, this enzyme displaces the equilibrium of the myokinase reaction toward adenosine triphosphate (ATP) production. The human *AMPD1* gene (location: 1p13) produces isoform M, myoadenylate deaminase, and is expressed at a high level predominantly in adult skeletal muscle.

Homozygotes for the rs17602729 mutation (34C > T; Gln12X) of the *AMPD1* have extremely low skeletal muscle AMPD activities, individuals with one normal and one mutant allele have intermediate activities, and those with two *AMPD1* normal alleles have high activities. Fischer et al. (2007) revealed a faster power decrease in the AMPD-deficient group during the 30-s Wingate cycling test. These data indicate that AMPD deficiency could have a detrimental effect on sprint/strength performance. Indeed, Cięszczyk et al. have shown that Polish power-oriented athletes have a significantly lower frequency of the *AMPD1* 12X allele than controls (Cieszczyk et al., 2012). These results were replicated in cohorts of Russian power-oriented athletes (Fedotovskaya et al., 2013a) and Lithuanian sprint and power athletes (Ginevičienė et al., 2014).

3.1.8 *ARHGEF28* rs17664695 G allele

This gene encodes a member of the Rho guanine nucleotide exchange factor family, defined by their ability to catalyze the exchange of guanosine diphosphate (GDP) for guanosine triphosphate (GTP) on small GTPase proteins such as Rho family members. The protein encoded by *ARHGEF28* gene (location: 5q13.2) promotes activity of Rho GTPases that control signaling pathways regulating cell proliferation and movement and in this way is engaged in cell division and growth (Miller et al., 2014).

During genome-wide association study (GWAS) of Russian athletes (strength athletes, endurance athletes, and athletes from other sports with a strength component) and controls, the rs17664695 G allele has been identified as being associated with elite strength athlete status (when compared with controls) (Egorova et al., 2015). The molecular basis of rs17664695 SNP is unknown and requires further studies.

3.1.9 *CACNG1* rs1799938 A allele (196Ser)

Voltage-dependent calcium channels are composed of five subunits. The protein encoded by *CACNG1* (location: 17q24.2) represents gamma1 subunit that is a muscle-specific isoform of the Ca^{2+} channel subunit and is an integral membrane protein that plays a role in excitation-contraction coupling.

The amino acids change of Gly to Ser in position 196 (located in transmembrane protein part) is a result of G to A SNP (rs1799938). The 196Ser allele has been recognized as significant and associated with elite strength athlete status in GWAS of Russian athletes (Egorova et al., 2015). However, the detailed impact of Gly196Ser substitution on strength properties needs to be established.

3.1.10 *CALCR* rs17734766 G allele

CALCR gene (location: 7q21.3) encodes a high-affinity receptor for the peptide hormone calcitonin. The activity of this receptor is mediated by G proteins which activate adenylyl cyclase. The stimulation of calcitonin receptor maintains calcium homeostasis and is involved in regulating osteoclast-mediated bone resorption.

Another GWAS performed by Ischenko et al. in Russian power athletes, group of athletes with speed/strength component, endurance athletes and controls (Ischenko et al., 2015) revealed that *CALCR* G allele of rs17734766 is associated with power athlete status. The genetic variation within *CALCR* gene has been correlated with bone mineral density and onset of osteoporosis (Taboulet et al., 1998). It might be speculated that this SNP would play a role in regulation of bone mass in humans. Thus, athletes carrying the specific form of *CALCR* gene might benefit from having stronger bones that are better adjusted to withstand extreme forces and transfer loads that are over the normal loading conditions. This aspect is especially important for athletes performing strength sports such as powerlifting or weightlifting, for which tremendous weight loads are transferred throughout the whole training program and during competition.

3.1.11 *CKM* rs8111989 G allele

Among the many specific genes and sequence variants within genes that have been associated with performance, the muscle-specific creatine kinase (*CKM*) gene (location: 19q13.32) is an important candidate gene due to its role in energy homeostasis in muscle cells. CKM catalyzes the reversible transfer of energy-rich phosphate from creatine phosphate to adenosine diphosphate, thus forming ATP. CKM is an essential enzyme for the maintenance of energy in the muscle cell during activity involving muscle contraction (Schneider et al., 1995).

The most frequently analyzed genetic variant of this gene is a polymorphism rs8111989 located in the 3′UTR. The A/G variants of this SNP have been associated with skeletal muscle performance in humans; they are correlated with physical performance and contribute to differences in the maximum oxygen uptake (VO_{2max}) responses during power or endurance training. The meta-analyses of five publications (Muniesa et al., 2010; Fedotovskaya et al., 2012, 2013b; Eider et al., 2015; He et al., 2016) on the *CKM* rs8111989 A/G allele or genotype differences between power and general

controls found that the power athletes had a significantly higher frequency of the G and GG genotype compared to controls (Chen et al., 2017).

3.1.12 *CLSTN2* rs2194938 C allele

CLSTN2 (location: 3q23) encodes the synaptic protein calsyntenin 2 that is part of the components of the postsynaptic membrane and is located predominately in excitatory synapses giving *CLSTN2* a role in intracellular postsynaptic signaling, potentially mediating specific responses in excitatory synaptic transmission (Hintsch et al., 2002).

By comparing genetic profiles of two groups of elite Russian athletes (elite power and endurance athletes), and then between power athletes and controls, Gabdrakhmanova et al. (2015) identified *CLSTN2* rs2194938 C allele as associated with power athlete status. Nevertheless, the detailed mechanism of rs2194938 SNP impact on athlete's organism still remains unclear.

3.1.13 *CNDP1* rs2887 A, rs2346061 C alleles, and *CNDP2* rs3764509 G allele

Carnosine (β-alanyl-L-histidine), is a dipeptide synthesized from b-alanine and histidine, found in the skeletal muscle, plays an important role during exercise, especially in muscles that rely on anaerobic metabolism to fuel their activity for high-intensity contractions.

Variability in muscle carnosine content between individuals exists and may be explained by different factors, for example, activity of carnosine-degrading enzymes named carnosine dipeptidases. A variation in carnosine dipeptidases activity could directly or indirectly affect muscle carnosine content (Everaert et al., 2011). Two forms of carnosine dipeptidases (and their genes in locus: 18q22.3) have been identified: CNDP1 (found in serum, encoded by *CNDP1*) and CNDP2 (tissue carnosine dipeptidase, encoded by *CNDP2*).

The study performed in a large Brazilian cohort of athletes revealed that three SNPs (*CNDP1* rs2887, rs2346061, and *CNDP2* rs3764509) were associated with power athlete status. The carriers of the rs2887 A allele, rs2346061 Callele, and rs3764509 G allele had an increased odds ratio of being a power athlete (Guilherme and Lancha, 2017). Such result suggests that polymorphisms in carnosine dipeptidases genes may be included into the group of power markers, however, this assumption needs a replication studies in different populations.

3.1.14 *CNTFR* rs41274853 T allele

Ciliary neurotrophic factor (CNTF) is expressed in glial cells within the central and peripheral nervous systems. CNTF stimulates gene expression, cell survival, or differentiation in a variety of neuronal cell types (Sleeman et al., 2000). All CNTF actions are triggered by binding to its receptor: CNTFR (Ip et al., 1993), which expression is relatively high in

skeletal muscle (Davis et al., 1991). Moreover, the analysis with use of the rat model revealed that the expression of *Cntfr* mRNA is greater in fast-twitch muscle than in slow-twitch muscle (Guillet et al., 1998). In mice with blocked *Cntfr* gene, a severe motor neuron deficit has been observed (DeChiara et al., 1995). These data suggested that CNTFR may contribute to neuromuscular development and maintenance.

The report of Miyamoto-Mikami et al. indicated that C/T substitution (rs41274853) in the *CNTFR* gene (location: 9p13.3) can influence sprint/power athletic status. The TT genotype was significantly more frequent among international sprint/power athletes than in the controls. Furthermore, in nonathletic men, TT genotype carriers exhibited significantly greater leg extension power and vertical jump performance (Miyamoto-Mikami et al., 2016). All these observations implicate that rs41274853 SNP may be associated with sprint/power athletes status, but without more comprehensive studies and confirmations of the trends in allele and genotypes frequencies, it will be rather a potential than a true marker.

3.1.15 *COTL1* rs7458 T allele

Human *COTL1* gene (location: 16q24.1) was identified as a filamentous actin (F-actin) binding protein which regulate the actin cytoskeleton. This protein also interacts with 5-lipoxygenase (5LO) binding partner, which is the first committed enzyme of leukotrien biosynthesis in the leukocytes (Provost et al., 2001).

In GWAS of Ischenko et al., the *COTL1* rs7458 T allele has been in the group of SNPs that were correlated with power athlete status and subsequently replicated in all three subgroups of power athletes (regardless of their level of achievement) (Ischenko et al., 2015). The molecular aspects of this SNP have not been yet described and further studies on the role of rs7458 alleles are required.

3.1.16 *CREM* rs1531550 A allele

The *CREM* gene (location: 10p11.21) encodes a cAMP-responsive element modulator which is a bZIP transcription factor that binds to the cAMP-responsive element found in many viral and cellular promoters. It is an important component of cAMP-mediated signal transduction during the spermatogenetic cycle, as well as other complex processes. *CREM* is highly expressed in testis, heart, brain, pancreas, and retina.

By performing three GWASes of elite Jamaican, African-American, and Japanese sprint athletes and their matched controls and subsequent meta-analysis, Wang et al. have found that the *CREM* A allele of the rs1531550 G/A polymorphism was significantly over-represented in elite sprinters compared with controls (Wang et al., 2014).

3.1.17 *DMD* rs939787 T allele

The dystrophin (*DMD*; location: Xp21.2) gene is the largest gene found in nature (2.4 Mb). Dystrophin RNA is differentially spliced, producing a range of different

transcripts, encoding a large set of protein isoforms. Dystrophin is a large, rod-like cytoskeletal protein which is found at the inner surface of muscle fibers. Dystrophin is part of the dystrophin-glycoprotein complex, which bridges the inner cytoskeleton (F-actin) and the extra-cellular matrix.

Since strength/power and endurance are located at the opposite extremes of a muscle performance continuum, a GWAS of elite Russian strength/power and endurance athletes was performed to identify common genetic variants associated with elite athlete status (Naumov et al., 2014). The comparison of genetic profiles of two groups of athletes and controls revealed that the rare *DMD* rs939787 T allele was over-represented in strength/power athletes compared to endurance athletes and controls, indicating that *DMD* rs939787 T allele is favorable for strength/power performance.

3.1.18 *EPAS1* rs1867785 G and rs11689011 C alleles

Endothelial PAS domain protein 1 (EPAS1) is a hypoxia-inducible transcription factor and plays an important role in the catecholamine and mitochondrial homeostasis, in the control of cardiac output and erythropoietin regulation.

Recently, Voisin et al. have investigated the frequencies of the *EPAS1* (also known as *HIF2A*; location: 2p21-p16) gene variants in sprint/power athletes, endurance athletes, and controls from Poland and Russia and found that the rs1867785 G and rs11689011 C alleles were over-represented in the sprint/power athletes (Voisin et al., 2014).

3.1.19 *FOCAD* rs17759424 C allele

FOCAD gene (location: 9p21.3) encodes focadhesin that is engaged in proliferation of epithelial cells and acts as a tumor suppressor (Brockschmidt et al., 2012). There are some reports raising the possibility that *FOCAD* is influencing heart rate via myocyte turnover and that the effect of *FOCAD* may change with age, either as a consequence or as a cause of the changing rate of cardiomyocyte renewal with age—however, the authors of these suggestion offer little evidence in this regard (Melton et al., 2010).

The analyses of genetic profiles performed between two groups of elite Russian athletes (power and endurance athletes) as well as between power athletes and controls, revealed that *FOCAD* rs17759424 C allele is associated with power athlete status (Gabdrakhmanova et al., 2015). In this moment, the physiological relevance of rs17759424 SNP is still unknown and more detailed studies are necessary.

3.1.20 Folate-pathway genetic markers (*MTHFR* rs1801131 C allele, *MTR* rs1805087 G allele and *MTRR* rs1801394 G allele)

DNA methylation is a major epigenetic modification that suppresses gene expression by modulating the access of the transcription machinery to the chromatin or by recruiting methyl-binding proteins. Barrès et al. (2012) have shown that exercise-induced acute

gene activation was associated with a dynamic change in DNA methylation in skeletal muscle and have suggested that DNA hypomethylation is an early event in contraction-induced gene activation. More specifically, whole genome methylation was decreased in skeletal muscle biopsies obtained from healthy sedentary men and women after acute exercise. Furthermore, recent findings suggest that DNA hypomethylation induces the activation of myogenic factors determining proliferation and differentiation of myoblasts promoting muscle growth and increase of muscle mass (Terruzzi et al., 2011).

Since components of the folate-pathway (homocysteine cycle) are involved in DNA methylation/demethylation processes (and synthesis of nucleotides), Terruzzi et al. have also investigated whether polymorphisms of the folate pathway genes affecting gene expression and protein stability, probably responsible of DNA methylation deficiency, are associated with athlete status. The polymorphic variants A1298C (rs1801131 A/C) of 5,10-methylenetetrahydrofolate reductase (*MTHFR*; location: 1p36.3), A2756G (rs1805087 A/G) of methionine synthase (*MTR*; location: 1q43), and A66G (rs1801394 A/G) of methionine synthase reductase (*MTRR*; location: 5p15.31) genes were determined in athletes and control subjects. The frequencies of *MTHFR* rs1801131 C, *MTR* rs1805087 G, and *MTRR* rs1801394 G alleles (probably associated with a reduced DNA methylating capacity) were significantly higher in athletes compared with controls (Terruzzi et al., 2011). Recently, Zarebska et al. have replicated the results of association between the *MTHFR* rs1801131 C allele and power/strength athlete status in a study of power/strength athletes from Poland and Russia (Zarebska et al., 2014). Taken together, these data indicate that elite athletes have a genetic predisposition to DNA hypomethylation and synthesis (factors leading to myogenic differentiation stimulation, muscle mass increase, and induction of genes involved in energy metabolism).

3.1.21 *GABRR1* rs282114 A allele

GABA is the major inhibitory neurotransmitter in the mammalian brain where it acts at GABA receptors, which are ligand-gated chloride channels. GABRR1 is a member of a family of such chloride channels and is one of the major inhibitory neurotransmitter receptors in the central nervous system (Cutting et al., 1991).

GABRR1 gene (location: 6q15) rs282114 A allele has been in the group of SNPs that were correlated with power athlete status in the GWAS of Russian athletes. All SNPs in this group have been characterized by an increase of the frequency of effect allele with increase of the level of achievement of strength athletes, significant differences in allelic frequencies between strength and endurance athletes and at least one replication of association between these alleles and predisposition to other sports with strength component (Egorova et al., 2015). Considering the physiological role of GABRR1 receptor, it might

be speculated that rs282114 SNP alters the neurotransmission process—however, it needs in vitro and in vivo confirmations.

3.1.22 *GALNT13* rs10196189 G allele

The protein encoded by *GALNT13* (location:2q24.1) is a member of the GALNT family, which initiate O-linked glycosylation of mucins by the initial transfer of N-acetylgalactosamine with an alpha-linkage to a serine or threonine residue and thus catalyzes the initial reaction in O-linked oligosaccharide biosynthesis. *GALNT13* is highly expressed in brain, B cells, kidney, and liver and may be involved in metabolism and energy pathways.

By performing three GWASes of elite Jamaican, African-American and Japanese sprint athletes and their matched controls and subsequent meta-analysis, Wang et al. (2014) have found that the *GALNT13* G allele of rs10196189 A/G polymorphism was significantly over-represented in elite sprinters compared with controls.

3.1.23 *GPC5* rs852918 T allele

Cell surface heparan sulfate proteoglycans are complex molecules present in the cell membrane and extracellular matrix, which play pivotal roles in cell adhesion, migration, proliferation, and signaling pathways. Mostly they are composed of one or more heparan sulfate chains covalently bound to the membrane-associated protein (Gardiner, 2017). GPC5 belongs to the GRIPS family consisted of glypican-related integral membrane proteoglycans that are linked to the cell surface via glycosyl phosphatidylinositol. These proteins may play a role in the control of cell division and growth regulation.

In the GWAS performed by Egorova et al., the *GPC5* gene (location: 13q32) rs852918 T allele was identified as associated with elite strength athlete status (Egorova et al., 2015), probably influencing the complicated processes of cell division and growth.

3.1.24 *HIF1A* rs11549465 T allele (582Ser)

Glycolysis is the central source of anaerobic energy in humans, and this metabolic pathway is regulated under low-oxygen conditions by the transcription factor hypoxia-inducible factor 1α (HIF1α; encoded by *HIF1A*; location: 14q23.2). HIF1α controls the expression of several genes implicated in various cellular functions including glucose metabolism (glucose transporters and glycolytic enzymes). A missense polymorphism, Pro582Ser, is present in exon 12 (C/T at bp 85; rs11549465). The rare T allele is predicted to result in a proline to serine change in the amino acid sequence of the protein. This substitution increases HIF1α protein stability and transcriptional activity, and therefore, may improve glucose metabolism.

Ahmetov et al. investigated a hypothesis that *HIF1A* Pro582Ser genotype distribution may differ for controls and Russian sprint/strength athletes, for which anaerobic glycolysis is one of the most important sources of energy for power performance. The frequency of the *HIF1A* 582Ser allele was significantly higher in weightlifters than in controls and increased with their levels of achievement (Ahmetov et al., 2008a). These results were replicated in three cohorts of Polish power-orientated athletes (Cięszczyk et al., 2011), Russian power-oriented athletes (Gabbasov et al., 2013), and Ukrainian power-oriented athletes (Drozdovska et al., 2013), but not in Israeli sprinters (Eynon et al., 2010b). Furthermore, the 582Ser allele was significantly associated with an increased proportion of fast-twitch muscle fibers in *M. vastus lateralis* of all-round speed skaters (Ahmetov et al., 2008a).

3.1.25 *HSD17B14* rs7247312 G allele

17-beta-hydroxysteroid dehydrogenases, such as HSD17B14, are primarily involved in metabolism of steroids at the C17 position and also of other substrates, such as fatty acids, prostaglandins, and xenobiotics (Sivik et al., 2012). Particularly, 17-beta-hydroxysteroid dehydrogenases catalyze the stereospecific oxidation/reduction at carbon 17β of androgens and oestrogens (Sherbet et al., 2009). Upon receptor binding, the 17β-hydroxy conformation of androgens and estrogens triggers a greater biological response than the corresponding keto-conformation of the steroids and, in this way, the HSD17B14 enzyme may be considered as an important mediator in pre-receptor regulation of sex hormone action (Sonneveld et al., 2006).

In the GWAS of Ischenko et al., the *HSD17B14* gene (location: 19q13.33) rs7247312 G allele has been annotated to the group of the markers associated with power athlete status along with the suggestion that it may be involved in alteration of steroids metabolism (Ischenko et al., 2015).

3.1.26 *IGF1* rs35767 T allele and *IGF1R* rs1464430 C allele

Insulin-like growth factor 1 (IGF1; encoded by *IGF1*; location: 12q23.2) plays an important role in growth and can induce hypertrophy of skeletal muscle and other target tissues. The insulin-like growth factor 1 receptor (IGF1R; encoded by *IGF1R*; location: 15q26.3) is a transmembrane receptor that mediates the effects of IGF1. Mice lacking the IGF1 receptor die late in development, and show a dramatic reduction in body mass, testifying to the strong growth-promoting effect of this receptor. Mice carrying only one functional copy of IGF1R are normal, but exhibit a ∼15% decrease in body mass. The minor T allele of the C-1245T (rs35767 C/T) polymorphism was found to be associated with higher circulating IGF1 levels, and possibly with increased muscle mass.

Ben-Zaken et al. (2013a) in a study of power athletes found that the *IGF1* rs35767 T allele was more frequent in the top-level Israeli power athletes (international and

Olympic level) compared to national level athletes. In the second study of Israeli power athletes, Ben-Zaken et al. (2015b) have shown that *IGF1R* C allele of the rs1464430 A/C polymorphism was more prevalent in elite power athletes in comparison with less professional power athletes or endurance athletes. The authors also concluded that the *IGF1R* AA genotype may be beneficial for endurance-type sports.

3.1.27 *IGF2* rs680 G allele

Insulin-like growth factor 2 (IGF2) is a protein hormone that is structurally similar to insulin, and act as growth-regulating, insulin-like, and mitogenic factor. The IGF2 is encoded by *IGF2* gene (location: 11p15.5). *IGF2* is maternally imprinted and paternally expressed what indicates its key role as a regulating factor in fetal growth and development. IGF2, along with IGF1, plays pivotal role in skeletal muscle growth and differentiation. Several SNPs of *IGF2* gene (rs3213221, rs680, rs7924316) were associated with loss of muscle strength directly after exertional muscle damage. The *IGF2* rs680 A/G polymorphism was associated with changes in the levels of *IGF2* mRNA. Particularly, significantly higher levels of *IGF2* mRNA were observed for G allele when compared with the A allele. It was then suggested that this polymorphism plays a role in the *IGF2* transcription.

The study by Ben-Zaken et al. (2017) that aimed to assess the frequency distribution of the *IGF2* rs680 A/G polymorphism among Israeli athletes showed that the frequency of G allele carriers was significantly greater among top compared to national-level track and field sprinters and jumpers. The rs680 GG genotype frequency was significantly greater among track and field sprinters and jumpers compared to weight lifters and among top-level sprinters and jumpers compared to top-level weight lifters.

3.1.28 *IL1RN* rs2234663 IL1RN*2 allele

Inflammation may serve as a mechanism promoting skeletal muscle repair and hypertrophy. Interleukin-1 receptor antagonist (IL-1RA) is a member of the interleukin 1 (IL-1) cytokine family and modulates a variety of IL-1-related immune and inflammatory responses. IL-1RA exerts anti-inflammatory activity by blocking IL-1 receptors and thereby preventing signal transduction of the pro-inflammatory IL-1. The IL-1RA is involved in the inflammatory and repair reactions in skeletal muscle during and after exercise (Pedersen, 2000).

The IL-1RA is encoded by the *IL1RN* gene (location: 2q14.2) in close proximity to the genes coding for IL-1α and IL-1β. The VNTR polymorphism (rs2234663) in intron 2 of the *IL1RN* gene is caused by the 86-bp variable copy number tandem repeat (two to six repeats), that contains three potential protein-binding sites and therefore, may have functional significance. The allele 1 (*IL1RN**1*) with four repeats is more common than allele 2 (*IL1RN**2*), containing two repeats. Alleles with three, five, and six repeats are

considered to be rare (<1%). In a recent study of Italian athletes and nonathletic controls, Cauci et al. (2010) have found that *IL1RN* gene VNTR polymorphism is associated with athletic status. The frequencies of the *IL1RN*1/IL1RN*2* genotype and *IL1RN*2* allele were significantly higher in athletes compared to nonathlete controls. Furthermore, the *IL1RN*1/IL1RN*2* genotype was more frequent in professional than in non-professional athletes. One might assume that carriers of the *IL1RN*2* allele may have an advantage in adaptation to high-intensity exercise.

3.1.29 *IL6* rs1800795 G allele

The interleukin-6 (IL-6) [also known as B-cell stimulatory factor-2 (BSF-2) and interferon beta-2] is a pleiotropic cytokine involved in a wide variety of biological functions, including regulation of differentiation, proliferation and survival of target cells, and control for the immune acute-phase response. It is mainly produced by the immune cells, but also is expressed in muscle cells (acts as a "myokine"), and is elevated in the response to muscle contraction (Febbraio and Pedersen, 2005). During physical exercise, the concentration of plasma IL-6 increases because of its release from muscles, which mediates metabolic processes. The IL-6 is linked to the regulation of glucose homeostasis during exercise and plays a role in the hypertrophic muscle growth with a contribution of satellite cells to this process (Serrano et al., 2008).

The -174 C/G (rs1800795) polymorphism in the promoter of the *IL6* gene (location: 7p21) alters transcriptional response. There is a genetically determined difference in the degree of the IL-6 response to stressful stimuli between individuals, with C allele found to be associated with significantly lower levels of plasma IL-6. Ruiz et al. (2010) studied the *IL6* -174 G/C polymorphism in elite Caucasian Spanish male athletes (endurance athletes and power athletes) and nonathletic controls. The frequencies of the GG genotype and G allele were significantly higher in power-oriented athletes compared with the endurance-oriented athletes and nonathletic controls. These results were replicated by Eider et al. (2013) in a study of Polish power athletes, indicating that G allele might favor sprint/power sports performance. Not consistent with results of the Spanish and Polish studies, Eynon et al. (2011) reported that there were no differences in allelic and genotypic frequencies of the *IL6* -174 C/G polymorphism among elite endurance athletes, power athletes, and nonathletic controls (Israeli population).

3.1.30 *IP6K3* rs6942022 C allele

This gene encodes a protein that belongs to the inositol phosphokinase (IPK) family. Inositol hexakisphosphate kinase 3 (IP6K3) generates inositol pyrophosphates (particularly diphosphoinositolpentakisphosphate), which regulate diverse cellular functions by controlling the cellular signaling and interact with other cellular components (Moritoh et al., 2016).

The GWAS performed by Ischenko et al. found that the *IP6K3* gene (location: 6p21.31) rs6942022 C allele was associated with power athlete status. Moreover, in the same study, these results were replicated in different subgroups of power athletes, indicating a real correlation existing with regard to this SNP (Ischenko et al., 2015). The analyses of mouse knockout models showed unchanged skeletal muscle mass and no resistance to the effects of high-fat diet (Moritoh et al., 2016), however, *Ip6k3*$^{-/-}$ mouse displayed neurological defects of motor learning and coordination (Fu et al., 2015). Further studies on the physiological role of rs6942022 SNP are required to establish its relevance for power athlete status.

3.1.31 *MCT1* rs1049434 T allele

MCT1 (location: 1p12) gene encodes monocarboxylate (lactate/pyruvate) transporter 1 (MCT1) that mediate, together with MCT4, the transmembrane cotransport of lactate and protons, relative to the lactate concentration and proton gradient, either into or out of skeletal muscle. MCT1 is more prevalence in type I oxidative muscle fibers.

A common A1470T (Glu490Asp) polymorphism (rs1049434) that leads to the replacement of glutamic acid with aspartic acid has been identified in the *MCT1* gene (location: 1p13.2). Carriers of the minor T allele have 60%–65% reduced lactate transport rates and experience higher blood lactate accumulations during high-intensity circuit weight training, compared to carriers of the A allele. In the study by Sawczuk et al., it was shown that *MCT1* T allele is associated with sprint/power performance in a recessive genetic model and the TT genotype was more prevalent in sprint/power athletes compared to both controls and endurance athletes, suggesting that *MCT1* A1470T might be one polymorphism that influence athletic sprint-power performance in Polish population (Sawczuk et al., 2015). However, this observation was not apparently confirmed in three studies involving Russian, Israeli, and Japanese power-oriented athletes (Fedotovskaya et al., 2014; Ben-Zaken et al., 2015a; Kikuchi et al., 2017).

3.1.32 *MED4* rs7337521 T allele

The protein encoded by *MED4* gene is a mediator complex subunit 4, which is a component of the vitamin D receptor-interacting protein (DRIP) complex functioning as a nuclear receptor coactivator. The DRIP complex interacts with DNA-binding gene-specific transcription factors to modulate transcription by RNA polymerase II and in this way is capable of activating nuclear receptors in a ligand-dependent manner. Binding to ligands induces conformational changes in the nuclear receptors that enable the nuclear receptors to interact with several types of coactivators, such as DRIP that are critical for transcription activation (Rachez et al., 1999).

Egorova et al. revealed that *MED4* gene (location: 13q14.2) rs7337521 T allele is associated with elite strength athlete status (Egorova et al., 2015). In this moment, there

is very limited information on the rs7337521 SNP with regard to its physiological or molecular relevance.

3.1.33 *MPRIP* rs6502557 A allele

The human *MPRIP* gene (location: 17p11.2) is encoding a myosin phosphatase Rho-interacting protein. This protein is known as a fusion partner of the receptor TK gene *NTRK1* (Vaishnavi et al., 2013). The MPRIP protein occurs together with actin myofilaments, and binds to myosin phosphatase and RhoA (Diekmann and Hall, 1995). A specific domain at the amino terminus of MPRIP has been shown to mediate actin binding in vivo (Mulder et al., 2003).

The genome-wide analyses revealed that *MPRIP* rs6502557 A allele is positively correlated with predisposition to sports with strength component, probably by influencing the muscle contraction (Egorova et al., 2015). Considering that this observation has not been replicated, it needs further investigation and conformation.

3.1.34 MtDNA markers

Mitochondria are essential to all higher organisms for sustaining life, and are extremely important in energy metabolism, providing 36 molecules of ATP per glucose molecule in contrast to the two ATP molecules produced by glycolysis. Although most DNA is packaged in chromosomes within the nucleus, mitochondria also possess their own circular DNA: mitochondrial DNA (mtDNA). The 16569-bp human mtDNA contains 13 genes for mitochondrial oxidative phosphorylation (OXPHOS), as well as two ribosomal RNA and 22 transfer RNA genes that are necessary for protein synthesis within mitochondria. Patients with mutations in mtDNA commonly display exercise intolerance, muscle weakness, and increased production of lactic acid.

At least four studies reported association between the 14 mtDNA polymorphisms and power athlete status. Mikami et al. (2011) analyzed mtDNA polymorphism in 139 Olympic athletes (79 endurance/middle-power athletes, 60 sprint/power athletes) and 672 controls. Sprint/power athletes displayed a greater proportion of haplogroup F (15.0% vs 6.0%; $P=0.007$). In a following study of 185 elite Japanese athletes and 672 controls, 85 sprint/power athletes showed greater frequency of the m.204C, m.151T, and m.15314A alleles and dearth of nine alleles (m.16278T, m.5601T, m.4833G, m.5108C, m.7600A, m.9377G, m.13563G, m.14200C, m.14569A) compared with controls (Mikami et al., 2013a, 2013b). Deason et al. (2012) revealed a high level of over-representation of the non-African component of mtDNA (non-L/U6 paragroup) in elite African-American sprinters ($n=119$) compared to African-American controls ($n=1148$).

3.1.35 *NOS3* rs2070744 T allele and rs1799983 G allele (Glu298)

Nitric oxide (NO) is involved in human skeletal muscle uptake during exercise and modulation of oxygen consumption in skeletal muscles. Dietary nitrate supplementation enhances muscle contractile efficiency during knee-extensor exercise and tolerance to high-intensity exercise in humans (Bailey et al., 2010).

Therefore, one might anticipate that genetic variation in the endothelial NO synthase gene (*NOS3*; location: 7q36; NOS3 generates NO in blood vessels) could be associated with power/sprint performance. Indeed, Drozdovska et al. (2009, 2013) have found that the frequency of the *NOS3* rs2070744 T (-786 T/C polymorphism) allele was significantly higher in Ukrainian power-oriented athletes compared to controls. These results were confirmed in two independent studies of Spanish elite power-oriented athletes and nonathletic controls (Gómez-Gallego et al., 2009) and Italian power-oriented athletes (Sessa et al., 2011). Furthermore, Sessa et al. have demonstrated that the frequency of the *NOS3* rs1799983 Glu298 allele (Glu298Asp polymorphism) was significantly higher in Italian power-oriented athletes in comparison with controls (Sessa et al., 2011).

3.1.36 *NRG1* rs17721043 A allele

The protein encoded by this gene (location: 8p12) is a membrane glycoprotein that mediates cell-cell signaling and plays a crucial role in the growth and development of organ systems as well as in the adult brain (Tan et al., 2007). The product of *NRG1* gene may be produced in many different isoforms due to the alternative promoter usage and splicing (Steinthorsdottir et al., 2004). The alterations of the *NRG1* function have been linked to diseases such as cancer, schizophrenia, and bipolar disorder (BPD) (Hahn et al., 2006; Walker et al., 2010; Fernandez-Cuesta and Thomas, 2015).

The GWAS study by Ischenko et al. has showed that *NRG1* rs17721043 A allele is associated with power athlete status (Ischenko et al., 2015). Considering the pleiotropic role of NRG1 protein, the functional relevance of rs17721043 SNP remains elusive.

3.1.37 *PPARA* rs4253778 C allele

PPARα is a ligand-activated transcription factor that regulates the expression of genes involved in fatty acid uptake and oxidation, glucose and lipid metabolism, left ventricular (LV) growth and control of body weight.

Jamshidi et al. have shown that British army recruits homozygous for the rare *PPARA* gene (location: 22q13.31) C allele of the rs4253778 (intron 7 G/C) polymorphism had a threefold greater increase in LV mass in response to training than G allele homozygotes (Jamshidi et al., 2002). The hypothesis that intron 7 C allele is associated with the hypertrophic effect due to influences on cardiac and skeletal muscle substrate utilization was supported by the findings that *PPARA* C allele is over-represented in Russian

power-oriented athletes and associated with an increased proportion of fast-twitch muscle fibers in *M. vastus lateralis* of male controls (Ahmetov et al., 2006). Furthermore, in a study of Lithuanian athletes, Ginevičienė et al. have shown that male athletes with *PPARA* CC and *PPARA* GC genotypes had significantly higher muscle mass and single muscular contraction power (measured by vertical jump test) than GG homozygotes. The frequency of the *PPARA* C allele was also significantly higher in Lithuanian power-oriented athletes and athletes with mixed aerobic/anaerobic activity in comparison with controls (Ginevičienė et al., 2010). It was also shown that the male carriers (middle school-age boys) of the *PPARA* C allele demonstrated the best results of handgrip strength testing than GG homozygotes (Ahmetov et al., 2013). However, Broos et al. did not find any association between the *PPARA* rs4253778 G/C polymorphism and muscle strength characteristics in nonathletic young men (Broos et al., 2013). There were no differences in allelic frequencies between Israeli sprinters and controls (Eynon et al., 2010a).

3.1.38 *PPARG* rs1801282 G allele (12Ala)

Peroxisome proliferator-activated receptor γ (PPARγ; encoded by *PPARG*; location: 3p25) plays a critical physiological role as a central transcriptional regulator of adipogenic and lipogenic programs, insulin sensitivity and glucose homeostasis.

The 12Ala variant of the *PPARG* gene Pro12Ala polymorphism (rs1801282 C/G) is associated with decreased receptor activity and improved insulin sensitivity (Deeb et al., 1998). The carriers of the 12Ala allele show higher rates of skeletal muscle glucose uptake (Vänttinen et al., 2005) and greater cross-sectional area of muscle fibers (Ahmetov et al., 2008b). In a study of Russian power-oriented athletes, the higher frequency of the *PPARG* 12Ala allele compared to controls has been reported (Ahmetov et al., 2008b). These results were replicated in two studies involving Polish strength athletes (Maciejewska-Karlowska et al., 2013) and Ukrainian power-oriented athletes (Drozdovska et al., 2013).

3.1.39 *PPARGC1A* rs8192678 A allele (482Ser) and *PPARGC1B* rs10060424 C allele

The PGC1α and PGC1β proteins (encoded by the*PPARGC1A*, location: 4p15.2 and *PPARGC1B* genes, location: 5q32, respectively) are a transcriptional coactivators of many different transcription factors and nuclear receptors. They act through direct interaction with transcription factor, control the energy expenditure and regulate fat oxidation as well as nonoxidative glucose metabolism (Liu and Lin, 2011). PGC1α and PGC1β are powerful regulators of mitochondrial biology in the heart, acting by broadly regulating gene expression from both nuclear and mitochondrial genomes—in this way, PGC1α and PGC1β play a critical role in mitochondrial metabolism. The allelic variations in their genes increase the risk of the development of two diabetes mellitus and obesity (Rowe et al., 2010).

It has been shown that *PPARGC1A* rs8192678 AA genotype is more favorable for Russian and Lithuanian powerlifters compared to controls (Gineviciene et al., 2016). However, this trend was not found in the previous study performed in the large group of Polish and Russian strength athletes (Maciejewska et al., 2012). Furthermore, in the GWAS study that has been planned to compare endurance vs power athletes and controls, the rs10060424 C allele of *PPARGC1B* gene has been designated as associated with power athlete status (Ischenko et al., 2015). It might be speculated that the importance of this two SNPs in power athletes results from its involvement in carbohydrate/lipid metabolism, however, the detailed mechanism of their molecular impact remains unknown.

3.1.40 *RC3H1* rs767053 G allele

RC3H1 gene (location: 1q25.1) encodes a protein containing RING-type and C3H1-type zinc finger motifs. The encoded protein, named ROQUIN, is involved in regulating mRNA translation and stability (Vinuesa et al., 2005). The mechanism of ROQUIN molecular action is based on recognition and binding to a constitutive decay element (CDE) in the 3′ UTR of mRNAs, leading to mRNA deadenylation and degradation (Yu et al., 2007). ROQUIN plays crucial role in innate and adaptative immune system by influencing macrophage function and the homeostasis of T and B cells. Moreover, the activity of ROQUIN is pivotal for T-cell-dependent B-cell response against infection. Impairment in ROQUIN activity results in autoimmunological diseases, for example, immunodeficiency (Athanasopoulos et al., 2016).

The *RC3H1* rs767053 G allele has been described as a marker for a power performance in GWAS of Russian athletes (Ischenko et al., 2015). The molecular mechanism of rs767053 impact on ROQUIN activity has not been yet described and in vitro studies are required.

3.1.41 *SOD2* rs4880 C allele (Ala16)

Manganese superoxide dismutase (MnSOD; encoded by the *SOD2* gene; location: 6q25.3) catalyzes the dismutation of superoxide radicals in mitochondria by converting anion superoxide into hydrogen peroxide and oxygen.

A number of polymorphisms in the *SOD2* gene have been described, and one polymorphism in the mitochondrial targeting sequence [rs4880 C/T; it causes an amino acid change from Ala (A) to Val (V)] has been demonstrated to have functional significance. Indeed, the T allele of the Ala16Val polymorphism in the *SOD2* gene has been reported to decrease MnSOD efficiency against oxidative stress (Shimoda-Matsubayashi et al., 1996), which is supported by the observation that the Val-variant decreases formation of the active MnSOD protein in the mitochondrial matrix (Sutton et al., 2003). Recently, Akimoto et al. demonstrated that athletes with the *SOD2* Val/Val genotype

had an increased CKM value after racing, suggesting that the Ala/Ala genotype is associated with lower muscle damage (Akimoto et al., 2010). In the first study involving Israeli power athletes, Ben-Zaken et al. found that the frequency of the *SOD2* Ala16 allele was significantly higher in athletes compared with controls (Ben-Zaken et al., 2013b). In a subsequent study of Russian and Polish athletes, the frequency of the *SOD2* Val/Val genotype was shown to be significantly lower in power/strength athletes compared to controls or athletes involved in low-intensity sports. Furthermore, the *SOD2* 16Val allele was significantly associated with increased activity of CKM and creatinine level in athletes (Ahmetov et al., 2014b).

3.1.42 *SUCLA2* rs10397 A allele

The *SUCLA2* gene (location: 13q14.2) encodes the beta-subunit of the ADP-forming succinyl-CoA synthetase (SCS). SCS is a mitochondrial matrix enzyme that acts as a heterodimer, being composed of an invariant alpha subunit and a substrate-specific beta subunit. SCS catalyzes the reversible synthesis of succinyl-CoA from succinate and CoA for activation of ketone bodies and heme synthesis (Chinnery, 2007). Defects in this gene are a cause of myopathic mtDNA depletion syndrome (El-Hattab and Scaglia, 2013).

The *SUCLA2* rs10397 A allele has been recognized as a marker for strength athlete status in the whole genome analyses performed by Egorova et al., even after applying the corrections criteria. The rs10397 variants are probably important for all athlete, regardless of discipline, because these variants may alter the regulation process of ATP production (Egorova et al., 2015). However, these speculations are not functionally confirmed, what indicate the need for further investigations.

3.1.43 *TPK1* rs10275875C allele

The protein encoded by this gene (location: 7q35) is a cellular enzyme involved in the regulation of thiamine metabolism. TPK1 functions as a homodimer and catalyzes the conversion of thiamine (a form of vitamin B1) to thiamine pyrophosphate, a cofactor for enzymes important in a range of fundamental processes such as cellular respiration, glycolytic and energy production pathways as well as in providing substrates for synthesis of nucleic acids, nucleotides, fatty acids, and steroids (Timm et al., 2001). A number of defects in thiamine transport and metabolism are known—in this group defects in *TPK1* gene are a cause of thiamine metabolism dysfunction syndrome-5 (Brown, 2014).

The comparison of genetic profiles of two groups of elite Russian athletes (power vs endurance and between power athletes and controls) identified *TPK1* rs10275875 C allele as a genetic marker associated with power athlete status (Gabdrakhmanova et al., 2015). The role of thiamin pyrophosphokinase 1 in vitamin B1 metabolism suggests that the variation of *TPK1* gene may be important for power athletes, because B vitamins are

involved in energy production and tissue repair and their normal metabolism is necessary for the appropriate recovery of the body after exercise.

3.1.44 *TRHR* rs7832552 T allele

The *TRHR* gene encodes a thyrotropin-releasing hormone (TRH) receptor (location: 8q23.1). Upon binding to TRH, this receptor activates the inositol phospholipid-calcium-protein kinase C transduction pathway. TRH is a releasing hormone, produced by the hypothalamus that stimulates the release of thyrotropin and prolactin from the anterior pituitary, thus having fundamental role in the regulation of metabolic and hormonal functions. Mutations in the *TRHR* gene have been associated with generalized TRH resistance. The *TRHR* rs7832552 T allele has been shown to be associated with increased lean body mass in four independent cohorts (Liu et al., 2009), as well as with elite sprint/power athlete status in Russian (Ahmetov et al., 2016) and Japanese (Miyamoto-Mikami et al., 2017) athletes.

3.1.45 *UCP2* rs660339 C allele (Ala55)

UCPs protein separate OXPHOS from ATP synthesis with energy dissipated as heat. Uncoupling protein-2 (UCP2) is one of the inner mitochondrial membrane proteins involved in energy expenditure that facilitates the transfer of anions from the inner to the outer mitochondrial membrane and the return transfer of protons from the outer to the inner mitochondrial membrane. *UCP2* gene (location 11q13.4) is expressed in many tissues, with the greatest expression in skeletal muscle.

The polymorphism C/T in *UCP2* gene resulting in Ala55Val amino acids substitution has been described as associated with power athletes status: the C allele (Ala55) was over-represented among Italian power athletes (Sessa et al., 2011).

3.1.46 *WAPL* rs4934207 C allele

WAPL protein is a cohesin-binding protein that promotes sister-chromatid resolution in mitotic prophase (Gandhi et al., 2006). Cohesin complexes are involved in arrangement of chromatin fibers into higher-order structures. It has been shown that depletion of the WAPL protein stably locks cohesin on DNA, leads to clustering of cohesin in axial structures, and causes chromatin condensation in interphase chromosomes. Furthermore, it has been revealed that regulation of cohesin-DNA interactions by WAPL is important for embryonic development and cell-cycle progression. In mitosis, WAPL-mediated release of cohesin from DNA is essential for proper chromosome segregation and protects cohesin from cleavage by the protease separase, thus enabling mitotic exit in the presence of functional cohesin complexes (Tedeschi et al., 2013).

In the GWAS of Russian athletes, encompassing strength and endurance athletes, it has been suggested that *WAPL* gene (location: 10q23.2) rs4934207 C allele is associated

with elite strength athlete status, what was confirmed in the same study after applying correction criteria (Egorova et al., 2015). However, to establish the role of rs4934207 SNP as a true marker of strength abilities, more detailed study of molecular as well as physiological aspects are required.

3.1.47 *ZNF423* rs11865138 C allele

The protein encoded by this gene (location: 16q12.1) is a nuclear protein that belongs to the family of Kruppel-like C2H2 zinc finger proteins. It functions as a DNA-binding transcription factor by using distinct zinc fingers in different signaling pathways. Thus, it is thought that this gene may have multiple roles in signal transduction during development (Harder et al., 2014). Moreover, it has been revealed that zinc finger protein 423 is a transcriptional regulator involved in preadipocyte determination. The molecular analysis revealed the ZFP423 protein as a factor enriched in preadipose vs non-preadipose fibroblasts (Gupta et al., 2010). The next step of differentiation of committed preadipocytes to adipocytes is controlled by PPARγ and several other transcription factors (Farmer, 2006). In mouse model, it has been shown that Zfp423 protein regulates *Pparg* expression. Furthermore, both brown and white adipocyte differentiation is markedly impaired in Zfp423-deficient mouse embryos (Gupta et al., 2010).

In the GWAS performed in a large group of Russian athletes *ZNF423* rs11865138 C allele has been described as a marker of power athlete status (Ischenko et al., 2015).

3.2 Conclusion

All aforementioned data provide evidence to support the notion that human physical performance might be influenced by genetic profiles, especially in power sports. Nevertheless, it needs to be highlighted that most of the cited above case-control and association studies have not yet been replicated in independent samples. Moreover, the problems of sample size, population stratification, and quality of the genotype/phenotype measurement are also of great importance. Furthermore, it must be emphasized that each DNA locus can probably explain a very small proportion of the phenotypic variance. Therefore, very large sample sizes are needed to detect associations and various combinatorial approaches (with use of rare mutations and epigenetics markers) should be considered. The current studies still represent only the first steps toward a better understanding of the genetic factors that influence power-related traits, so further research is necessary before implementation of research findings into practice.

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CHAPTER FOUR

Genetic profile of elite endurance athletes

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4.1 Introduction

Endurance performance is a complex phenotype, influenced by a number of factors, many relating to cellular metabolism and cardiovascular function (Moore, 1998; Bassett and Howley, 2000; Flück, 2006). These include the proportion of slow-twitch fibers in skeletal muscle, hemoglobin mass, maximal cardiac output, maximal rate of oxygen consumption (VO_{2max}), and others (Abernethy et al., 1990; Bassett and Howley, 2000; Malczewska-Lenczowska et al., 2016; Simoneau and Bouchard, 1995). Such intermediate phenotypes are under strong genetic influence. Indeed, studies indicate that genetic factors account for 44%–68% of the variability in endurance-related phenotypes (Miyamoto-Mikami et al., 2018).

Case-control studies remain the most common study design in genomics of endurance performance and generally involve determining whether one allele of a DNA sequence (gene or noncoding region of DNA) is more common in a group of elite endurance athletes than it is in the general population, thus implying that the allele boosts endurance performance (Ahmetov and Fedotovskaya, 2015). To avoid false-positive results, case-control studies should have at least one replication with additional athletic and nonathletic cohorts from different populations (external replication) or subgroups of the same cohort (internal replication) (Wang et al., 2013a; Ahmetov and Fedotovskaya, 2015). Another way to identify endurance-related genetic markers is to compare genotype and allelic frequencies between athletes with the best and the worst competition results (O'Connell et al., 2011; Brown et al., 2011b).

Since endurance and power are located at opposite extremes of the muscle performance continuum, the comparison of allelic or genotype frequencies between endurance and power/strength athletes is also the way to identify endurance markers (Drozdovska et al., 2013; Ahmetov et al., 2014). Cross-sectional association studies are another type of

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study design in sports genomics, and examine whether athletes with one genotype (or allele) of a particular DNA sequence have different measures of a trait (e.g., VO_{2max}, running time, percentage of slow-twitch muscle fibers, lactate levels, etc.) compared to the rest of the sample (Mustafina et al., 2014; Ahmetov et al., 2016).

A genome-wide association study (GWAS) is an approach that involves rapidly scanning several hundred thousand (up to 5 million in one microchip) markers across the complete sets of DNA by microchips of many people to find DNA polymorphisms associated with a particular trait. One of the advantages of the GWAS approach is that it is unbiased with respect to genomic structure and previous knowledge of the trait (hypothesis-free), in contrast to candidate gene studies, where knowledge of the trait is used to identify candidate loci contributing to the trait of interest (Wang et al., 2013a; Ahmetov et al., 2015b). Thus, GWASs facilitated by high-throughput genotyping technologies have been enormously successful in identifying single-nucleotide polymorphisms (SNPs) that are associated with complex traits.

According to the existing data, endurance athlete status remains the most studied trait in sports genomics. A literature search revealed that at least 100 endurance-related genetic markers [located within 89 autosomal genes, mitochondrial DNA (mtDNA), X- and Y-chromosomes] are linked to elite athlete status (Table 4.1). Of note, 23 genetic markers (*ACE* I, *ACTN3* 577X, *ADRB2* 16Arg, *AQP1* rs1049305 C, *AMPD1* Gln12, *BDKRB2* −9, *COL5A1* rs12722 T, *GABPB1* rs12594956 A and rs7181866 G, *GALNTL6* rs558129 G, *GSTP1* rs1695 G, *HFE* 63Asp, *KCNJ11* Glu23, *MCT1* Glu490, mtDNA H haplogroup, mtDNA K haplogroup (unfavorable), *NACC2* rs4409473 C, *PPARA* rs4253778 G, *PPARD* rs2016520 C, *PPARGC1A* Gly482, *VEGFR2* 472Gln, *UCP2* 55Val, *UCP3* rs1800849 T) have shown positive associations with athlete status in at least two studies and six of them (*ACE* I, *ACTN3* 577X, *HFE* 63Asp, *NACC2* rs4409473 C, *PPARA* rs4253778 G, *PPARGC1A* Gly482) in three or more studies. Conversely, the significance of 25 markers was not replicated in at least one study (for details, also see Ahmetov and Fedotovskaya, 2015), raising the possibility that several findings might be false-positive and require additional studies. Interestingly, almost all chromosomes (except for the chromosome 20) include sport-related genetic markers.

Due to space limitations, there was no possibility to describe all 100 DNA polymorphisms in this chapter; but it should be noted that most of these genetic markers (mainly identified by candidate gene approach) were comprehensively characterized in a recent review (Ahmetov and Fedotovskaya, 2015). Given that, in this chapter, we have focused on the description of DNA polymorphisms identified by the GWAS approach, as well as novel markers (published in 2017–18) and those markers which have shown positive associations with athlete status in at least two studies.

Table 4.1 Gene variants (genetic markers) for endurance athlete status

				Studies with positive results		Studies with negative or controversial results	
Gene	**Location**	**Polymorphism**	**Endurance-related marker**	**Number of studies**	**Total number of studied athletes**	**Number of studies**	**Total number of studied athletes**
ACE	17q23.3	Alu I/D (rs4646994)	I	16	1310	12	1329
ACOXL	2q13	rs13027870A/G	G	1	103	–	–
ACTN3	11q13.1	R577X (rs1815739 C/T)	577X	4	560	14	3039
ADRA2A	10q24-q26	6.7/6.3 kb	6.7-kb	1	148	–	–
ADRB1	10q25.3	Ser49Gly (rs1801252 A/G)	49Gly	1	124	–	–
ADRB2	5q31-q32	Gly16Arg (rs1042713 G/A)	16Arg	2	629	1	123
ADRB3	8p12-8p11.1	Trp64Arg (rs4994 T/C)	64Arg	1	100	1	81
AGT	1q42.2	Met268Thr (rs699 A/G)	Met268	1	328	–	–
AGTR2	Xq22-q23	rs11091046 A/C	C	1	487	1	316
AQP1	7p14	rs1049305 C/G	C	2	1288	–	–
AMPD1	1p13	Gln12X (rs17602729 C/T)	Gln12	2	231	1	84
BDKRB2	14q32.1-q32.2	+9/−9 (exon 1)	−9	2	524	3	408
		rs1799722 C/T	T	1	316	–	–
CAMK1D	10p13	rs11257754A/G	A	1	223	–	–
CHRNB3	8p11.21	rs4950 A/G	G	1	225	–	–
CKM	19q13.32	rs8111989 A/G	A	1	176	3	581
CLSTN2	3q23	rs2194938 A/C	A	1	223	–	–
CNDP2	18q22.3	rs6566810 A/T	A	1	117	–	–
COL5A1	9q34.2-q34.3	rs12722C/T	T	2	385	–	–
		rs71746744 (AGGG/−)	AGGG	1	106	–	–
COL6A1	21q22.3	rs35796750 T/C	T	1	661	–	–
CPQ	8q22.2	rs6468527A/G	A	1	223	–	–
	2p21-p16	rs1867785 A/G	G	1	451	1	254

Continued

Table 4.1 Gene variants (genetic markers) for endurance athlete status—cont'd

Gene	Location	Polymorphism	Endurance-related marker	Studies with positive results		Studies with negative or controversial results	
				Number of studies	Total number of studied athletes	Number of studies	Total number of studied athletes
EPAS1 (*HIF2A*)		rs11689011 C/T	T	1	451	1	254
GABPB1	15q21.2	rs12594956 A/C	A	2	163	–	–
		rs8031031 C/T	T	1	74	1	89
		rs7181866 A/G	G	2	129	1	89
GALM	2p22.1	rs3821023A/G	A	1	103	–	–
GALNTL6	4q34.1	rs558129A/G	G	2	275	8	1245
GNB3	2p13	rs5443 C/T	T	1	74	3	283
GRM3	7q21.1-q21.2	rs724225A/G	G	1	223	–	–
GSTP1	11q13.2	Ile105Val (rs1695A/G)	105Val	2	489	–	–
HFE	6p21.3	His63Asp (rs1799945 C/G)	63Asp	3	277	–	–
HIF1A	14q23.2	Pro582Ser (rs11549465 C/T)	Pro582	1	316	1	265
IGF1R	15q26.3	rs1464430 A/C	A	1	77	–	–
IL6	7p15.3	rs1800795 G/C	C	1	38	–	–
IL15RA	10p15.1	Asn146Thr (rs2228059 A/C)	Asn146	1	73	–	–
ITPR1	3p26.1	rs1038639G/T	T	1	223	–	–
		rs2131458C/T	T	1	103	–	–
FMNL2	2q23.3	rs12693407A/G	G	1	103	–	–
KCNJ11	11p15.1	Glu23Lys (rs5219 C/T)	Glu23	2	282	–	–
L3MBTL4	18p11.31	rs17483463C/T	T	1	223	–	–

MCT1	1p12	Glu490Asp (rs1049434 A/T)	Glu490	2	179	2	211
MtDNA loci	MtDNA	Haplogroups constructed from several MtDNA polymorphisms or single polymorphisms	G1	1	79	–	–
			H	2	143	–	–
			HV	1	91	–	–
			L0	1	70	–	–
			M*	1	75	–	–
			m.11215T, m.152C, m.15518T, m.15874G, m.4343G, m.514 $(CA)_{\leq 4}$, poly(C $\geq$ 7) stretch at m.568–573	1	100	–	–
			m.16080G	1	210	–	–
			m.5178C	1	66	–	–
			N9	1	75	–	–
			V	1	102	–	–
			Unfavorable: B	1	75	–	–
			Unfavorable: K	2	182	–	–
			Unfavorable: J2	1	52	–	–
			Unfavorable: T	1	95	–	–
			Unfavorable: L3*	1	70	–	–
NALCN-AS1	13q33.1	rs4772341 A/G	A	1	223	–	–
NACC2	9q34.3	rs4409473 T/C	C	4	459	–	–
NATD1	17p11.2	rs732928A/G	G	1	103	–	–
NFATC4	14q11.2	Gly160Ala (rs2229309 G/C)	Gly160	1	694	–	–
NFIA-AS2	1p31.3	rs1572312 C/A	C	1	218	–	–
NOS3	7q36	Glu298Asp (rs1799983 G/T)	Glu298	1	443	–	–
		$(CA)_n$ repeats	164-bp	1	316	–	–
		27 bp repeats (4B/4A)	4B	1	168	–	–

Continued

Table 4.1 Gene variants (genetic markers) for endurance athlete status—cont'd

Gene	Location	Polymorphism	Endurance-related marker	Studies with positive results		Studies with negative or controversial results	
				Number of studies	Total number of studied athletes	Number of studies	Total number of studied athletes
		rs2070744 T/C (−786 T/C)	T	1	71	1	100
PPARA	22q13.31	rs4253778 G/C	G	5	740	–	–
PPARD	6p21.2-p21.1	rs2016520 T/C	C	2	683	1	120
		rs1053049 T/C	T	1	120	–	–
PPARGC1A	4p15.1	Gly482Ser (rs8192678 G/A)	Gly482	4	849	3	508
		rs4697425A/G	A	1	127	1	194
PPARGC1B	5q32	Ala203Pro (rs7732671 G/C)	203Pro	1	578	–	–
		Arg292Ser (rs11959820 C/A)	292Ser	1	316	–	–
PPP3CA	4q24	rs3804358 C/G	C	1	123	1	100
PPP3CB	10q22.2	rs3763679 C/T	C	1	123	1	100
PPP3R1	2p15	Promoter 5I/5D	5I	1	694	–	–
RBFOX1	16p13.3	rs7191721 G/A	G	1	218	–	–
SGMS1	10q11.2	rs884880A/C	A	1	223	–	–
SLC2A4	17p13	rs5418 G/A	A	1	102	–	–
SOD2	6q25.3	Ala16Val (rs4880 C/T)	Ala16	1	121	1	508
SPOCK1	5q31.2	rs1051854G/T	T	1	223	–	–
TFAM	10q21	Ser12Thr (rs1937 G/C)	12Thr	1	588	1	213
TPK1	7q34-q35	rs10275875 C/T	T	1	223	–	–
TSHR	14q31	rs7144481 T/C	C	1	218	–	–

TTN	2q31.2	rs10497520	T	1	141	–	–
UCP2	11q13	Ala55Val (rs660339 C/T)	55Val	2	874	–	–
UCP3	11q13	rs1800849 C/T	T	2	877	1	178
VEGFA	6p12	rs2010963 G/C	C	1	942	–	–
VEGFR2	4q11-q12	His472Gln (rs1870377 T/A)	472Gln	2	358	–	–
Y-chromosome haplogroups	Y-chromosome	Haplogroups constructed from several Y-chr. polymorphisms	E*, E3* and K*(xP)	1	44	–	–
			Unfavorable: E3b1	1	44	–	–
ZNF429	19p12	rs1984771A/G	G	1	103	–	–

4.2 Gene variants identified in GWASs

A literature search revealed at least 21 genetic markers for endurance athlete status have been discovered by the use of microchip technology. Initially, Ahmetov et al. (2015b) examined the association between 1,140,419 SNPs and relative maximal oxygen consumption rate ($\dot{V}O_{2max}$) in 80 international-level Russian endurance athletes (46 males and 34 females), and identified 6 suggestive "endurance alleles" (with $P<10^{-5}$–10^{-8}) which were replicated both in female and male subgroups. To validate obtained results, the authors further performed case-control studies by comparing the frequencies of six SNPs between 218 endurance athletes (or 100 elite endurance athletes) and opposite cohorts (192 Russian controls, 1367 European controls, and 230 Russian power athletes). It was assumed that the "endurance allele" should be under-represented in at least one opposite cohort (Russian controls or Russian power athletes) when compared to endurance athletes. This approach resulted in the remaining three SNPs (*NFIA-AS2* rs1572312 C, *TSHR*rs7144481 C, *RBFOX1*rs7191721 G) associated with endurance athlete status.

The nuclear factor I A *(NFIA)-AS2* gene encodes long noncoding RNA (lnc-RNA) with undescribed function. Genes of antisense lnc-RNAs are transcribed from either the same genomic site or a site distant from the gene locus where the sense transcript counterpart is produced. Antisense lnc-RNAs repress—and in some cases can also activate—transcription of the targeted protein coding genes via mechanisms such as DNA methylation and chromatin modification at the genomic loci of the targeted genes. It was hypothesized that NFIA-AS2 is involved in the regulation of expression of the *NFIA* gene or erythroid/myeloid-specific RNAs. NFIA, as a transcription factor, induces erythropoiesis, whereas its silencing drives granulopoiesis. Consistent with the hypothesis, the authors also reported that the C allele was associated with activation of erythropoiesis (high level of hemoglobin, high number of reticulocytes, and erythrocytes), while the A allele with activated granulopoiesis (high number of neutrophils and greater leukocyte/erythrocyte ratio) (Ahmetov et al., 2015b).

As to the other two gene polymorphisms, it was established that RNA-binding protein, fox-1 homolog (*Caenorhabditis elegans*) 1 (encoded by *RBFOX1* gene) is an important splicing factor regulating developmental and tissue-specific alternative splicing in heart, muscle, and neuronal tissues (Kuroyanagi, 2009). Therefore, RBFOX1 is implicated in multiple medical conditions, including muscular dystrophies, cancers, neurodevelopmental, and neuropsychiatric disorders. The thyroid-stimulating hormone receptor encoded by *TSHR* gene is a membrane receptor for thyrotropin (produces thyroid hormones) and thyrostimulin (activates TSHR protein), and therefore, a major controller of thyroid cell metabolism. Thyroid hormones are known as determinants of metabolic and contractile phenotype of skeletal muscle (Simonides and van Hardeveld, 2008). TSHR also mediates

the effect of thyrotropin on angiogenesis via cAMP-mammalian target of rapamycin signaling (Balzan et al., 2012). The rs7144481 polymorphism is located in the regulatory region (3′UTR) of the *TSHR* gene.

The same group of authors in respect to the same groups of athletes and GWAS data using different criteria, such as: (i) the SNP should be independently associated with $\dot{V}O_{2max}$ in male and female athletes separately (with $P<10^{-3}$ adjusted for sex); (ii) the frequency of the endurance-related allele should be over-represented in endurance athletes in comparison with controls; and (iii) power athletes, identified additional six alleles (*ZNF429* rs1984771 G, *FMNL2* rs12693407 G, *ACOXL* rs13027870 G, *ITPR1* rs2131458 A, *GALM* rs3821023 A, *NATD1* rs732928 G) with suggestive significance in the determination of endurance performance (Ahmetov et al., 2015a). These SNPs are located in the genes involved in the regulation of lipid (*ACOXL*) and carbohydrate (*GALM*) metabolism, morphogenesis and cytokinesis (*FMNL2*), intracellular Ca^{2+} signaling (*ITPR1*), and other processes (*ZNF429, NATD1*).

In the third study of the same group, Galeeva et al. (2015) performed GWAS in four subgroups of Russian endurance athletes ($n=223$; all and elite long-distance athletes, all and elite middle distance athletes) and controls ($n=173$), and found 93 SNPs associated with endurance athlete status with replications in all subgroups ($P<10^{-4}$) but none of them reached genome-wide significance level. Adding three criteria, (i) an increase of the frequency of effect allele with increase of the level of achievement of endurance athletes; (ii) significant differences in allelic frequencies between 56 elite endurance athletes and 67 elite power athletes (second case-control study); and (iii) positive correlation of the effect allele with high values of $\dot{V}O_{2max}$, resulted in the remaining five SNPs (effect alleles: *CAMK1D* rs11257754 A, *CPQ* rs6468527 A, *GRM3* rs724225 G, *SGMS1* rs884880 A, *L3MBTL4* rs17483463 T) associated with elite endurance athlete status. These SNPs are located in the genes involved in the regulation of carbohydrate metabolism (*CAMK1D*), synthesis of thyroxine (*CPQ*), glutamatergic neurotransmission (*GRM3*), sphingomyelin and diacylglycerol metabolism (*SGMS1*), and chromatin modification (*L3MBTL4*).

Gabdrakhmanova et al. (2015) have studied the differences in genomic profiles between Russian endurance and power athletes using the GWAS approach. At the first stage, by comparing genetic profiles of two groups of elite athletes (171 elite power and 56 elite endurance athletes), the authors identified 13 SNPs with suggestive significance (*P* values from 10^{-5} to 10^{-6}). At the second stage, they compared allelic frequencies of the discovered SNPs between 223 endurance athletes and 173 controls. As a final point, the regression analysis was performed to reveal association with VO_{2max} of endurance athletes ($n=71$). These analyses resulted in the remaining five SNPs (*CLSTN2* rs2194938 A, *TPK1* rs10275875 T, *ITPR1* rs1038639 T, *NALCN-AS1* rs4772341 A, *SPOCK1* rs1051854 T) associated with endurance athletes status (based

on the case-control study and correlation with VO_{2max}). These SNPs are located in the genes involved in the regulation of neuronal excitability (*CLSTN2, NALCN-AS1*), vitamin B1 metabolism (*TPK1*), muscle contraction (*ITPR1*), and protein metabolism (*SPOCK1*).

In the first multi-ethnic collaborative GWAS, Rankinen et al. (2016) using a panel of 45 promising markers performed a meta-analysis in eight cohorts [GENATHLETE (Germany, the United States/Canada, Finland populations), Japan, Australia, Poland, Russia, Spain, Kenya, and Ethiopia). The study was based on a total of 1520 endurance athletes (835 who took part in endurance events in World Championships and/or Olympic Games) and 2760 controls. Only one marker, namely *GALNTL6* rs558129 G allele, has shown positive association ($P=0.0002$) with endurance athlete status after correcting for multiple testing. More specifically, this allele was found to be significantly over-represented in Japanese ($n=60$, $P=3.0\times10^{-5}$) and Australian ($n=215$, $P=0.01$) endurance athletes compared to controls, but not in other ethnicities although all eight cohorts showed the same direction of association with rs558129. The *GALNTL6* gene encodes N-acetylgalactosaminyltransferase-like 6, which plays a role in the initial reaction in O-linked oligosaccharide biosynthesis.

In the most recent GWAS involving Russian, Japanese, European, and African endurance athletes, Semenova et al. (2018) have identified a highly replicable SNP in the *NACC2* gene. In the discovery phase, 15 gene polymorphisms associated with endurance athlete status in both Russian (232 athletes) and Japanese (60 athletes) populations were identified. In the replication phase, the association of *NACC2* rs4409473 C allele with endurance athlete status was confirmed in Western European ($n=162$) and African cohorts ($n=24$) [meta-analysis: $OR=2.15$ (95% CI: 1.68–2.74), $P=7.8\times10^{-10}$]. *NACC2* encodes a transcriptional repressor which decreases the expression of the *MDM2*, an essential regulator of skeletal muscle angiogenesis. Indeed, prolonged exercise training results in increased MDM2 protein expression (+49%) and capillarization (+24%) in the muscles of mice (Roudier et al., 2012). There is a strong relationship between muscle capillarity, oxidative capacity, and maximal oxygen consumption rate ($\dot{V}O_{2max}$), factors which positively influence endurance performance. The C allele of the rs4409473 was previously reported as a variant with low *NACC2* expression (Fehrmann, 2011). Additional functional analyses revealed the association of CC genotype with increased skeletal muscle capillary density in Swedish untrained men ($n=656$), high values of $\dot{V}O_{2max}$ in Russian athletes ($n=34$), and untrained Japanese ($n=406$) subjects, high self-reported tolerance to long distances in endurance athletes ($n=21$), as well as with greater increase of $\dot{V}O_{2max}$ in Polish women ($n=93$) following a 12-week aerobic training (Semenova et al., 2018).

4.3 Novel markers for endurance athlete status

This section covers the research advances reported in the period between 2017 and 2018 (six novel genetic markers).

4.3.1 *AGT* rs699 Met268 allele

The angiotensinogen (AGT) (serpin peptidase inhibitor, clade A, member 8), serum α-globulin formed by the liver, is an essential component of the renin-angiotensin system. The AGT is cleaved by the renin to form biologically inactive angiotensin I, the precursor of active angiotensin II that regulates vascular resistance and sodium homeostasis, and thus determining blood pressure. The AGT is encoded by *AGT* gene (location: 1q42.2). The rs699 A/G polymorphism of the *AGT* gene leads to the substitution of methionine to threonine at position 268 (Met268Thr). The *AGT* rs699 polymorphism was shown to be associated with left-ventricular mass index increase in a study of 83 young healthy individuals after 17 weeks of exercise training (50%–80% $\dot{V}O_{2max}$) (Alves et al., 2009). Individuals with the *AGT* Thr/Thr genotype had significantly greater left-ventricular mass index (not favorable condition in terms of cardiovascular adaptation to aerobic exercise) than those with the Met/Met or Met/Thr genotype. Recently, Guilherme et al. (2018) have shown that *AGT* Met268 allele is associated with the endurance status: the carriers of the *AGT* Met/Met and Met/Thr genotypes had a greater likelihood of being an endurance athlete than carriers of the Thr/Thr genotype ($OR=0.68$, $P=0.028$). The Met/Met genotype was also over-represented in the endurance group (22.4%) compared with the power group (14.9%; $OR=1.65$, $P=0.015$).

4.3.2 *CHRNB3* rs4950 G allele

The *CHRNB3* gene (location: 8p11.21) encodes a neuronal acetylcholine receptor subunit beta-3. The members of this family of ligand-gated ion channels play an important role in fast signal transmission at synapses. Azam et al. (2002) have shown that subunit alpha-6 (encoded by gene *CHNRA6*) and beta-3 (*CHRNB3* gene) modulate dopamine release in the midbrain, which play a key role in positive reinforcement associated with acquired behaviors. De Neve et al. (2013) have reported that leadership is associated with the rs4950 A/G polymorphism in the *CHRNB3* gene. Motivation and achieve leadership position is important in sport. Peplonska et al. (2017) have shown that the frequencies of rs4950 GG+AG genotypes (50.7%) were significantly higher in endurance athletes ($n=225$) compared to the power group ($n=188$, 36.7%, $OR=1.77$, $P=0.0044$) or controls ($n=451$, 41.9%, $OR=1.42$, $P=0.031$).

4.3.3 *CNDP2* rs6566810 A allele

Carnosinase plays a role in the hydrolysis of carnosine (β-alanyl-L-histidine), which helps during exercise, especially for high-intensity contractions. There are two forms of carnosinase: serum carnosinase (CNDP1) and tissue carnosinase (CNDP2). A carnosinase-2 (*CNDP2*) gene (location: 18q22.3) is expressed in the skeletal muscle (Everaert et al., 2013). Guilherme and Lancha (2017) have reported that the *CNDP2* rs6566810 A/A genotype is over-represented in 117 Brazil international-level endurance athletes (11.1%) compared with 915 nonathletes (5.2%) and 408 power athletes (4.2%; recessive model; $P<0.05$). The authors have hypothesized that glucose/insulin metabolism is more efficient in individuals with higher blood carnosine, which may be important for endurance performance. However, the biological impact of the polymorphism and its significance in sports should be defined in the subsequent studies.

4.3.4 *GSTP1* rs1695 105Val allele

The *GSTP1* gene (location: 11q13.2) encodes a glutathione S-transferase P1, a member of large enzyme families, that play an important role in detoxification and antioxidant system. The rs1695 A/G polymorphism in the *GSTP1* gene leads to the Ile105Val amino acid change, which can substantially alter the GSTP1 activity. Matsui et al. (2000) have suggested that the Val/Val genotype carriers of the *GSTP1* gene would probably have lower levels of oxidative DNA damage. In the joint study of Russian ($n=207$) and Polish ($n=282$) endurance athletes, Zarebska et al. (2017) have identified the association of *GSTP1* Val allele with endurance athlete status. More specifically, the frequencies of the Val/Val genotype were significantly higher in Russian (8.7% vs 3.4%; $OR=2.72$, $P=0.0022$) and Polish (12.1% vs 6.4%; $OR=1.99$, $P=0.0035$) endurance athletes compared to controls. These results are in agreement with the observation from the previous study where the *GSTP1* Val allele was found to be associated with greater gains in VO_{2max} in response to aerobic training (Zarebska et al., 2014).

4.3.5 *IL6* rs1800795 C allele

The interleukin-6 (IL-6) (also known as B-cell stimulatory factor-2 (BSF-2) and interferon beta-2) is a pleiotropic cytokine involved in a wide variety of biological functions, including regulation of differentiation, proliferation and survival of target cells, and control for the immune acute-phase response. It is mainly produced by the immune cells, but also is expressed in muscle cells (acts as a "myokine"), and is elevated in the response to muscle contraction (Febbraio and Pedersen, 2005). During physical exercise, the concentration of plasma IL-6 increases because of its release from muscles and may be involved in the development of fatigue. The IL-6 is linked to the regulation of glucose homeostasis during exercise and plays a role in the hypertrophic muscle growth with a contribution of satellite cells to this process (Serrano et al., 2008).

The G allele of the rs1800795 C/G polymorphism in the *IL6* gene (location: 7p21) is associated with increased IL6 levels. Therefore, one might hypothesize that the G allele is unfavorable for endurance performance. Indeed, in the recent study of Israeli athletes, Ben-Zaken et al. (2017) have shown that the frequencies of the CC (18%) genotype and C (43%) allele were significantly higher in the long-distance swimmers compared to controls (CC genotype: 5%, $P<0.001$; C allele: 19%, $P<0.001$) and short distance swimmers (CC genotype: 4%, $P<0.001$; C allele: 29%, $P<0.05$).

4.3.6 *TTN* rs10497520 T allele

The titin (encoded by *TTN* gene; location: 2q31.2) is a structural protein that regulates assembly and organization of muscle filaments during myofibrillogenesis in the striated muscle. It was suggested that this protein may play a key role in the regulation of length of muscle fascicles (Greaser and Pleitner, 2014; Greaser et al., 2008). Timmons et al. (2010) have shown that the C allele of the rs10497520 C/T polymorphism of the *TTN* gene was associated with greater $\dot{V}O_{2max}$ gains in response to aerobic training ($P=0.0025$). Contrary to these observations, Stebbings et al. (2018) have found that the rs10497520 T allele was associated with shorter skeletal muscle fascicle length in recreationally active men ($n=137$) and with greater personal best times in male marathon runners ($n=141$; $P=0.020$).

4.4 Most studied markers

The most studied (in at least two studies) genetic markers are reviewed in this section except for *GALNTL6* rs558129 G, *GSTP1* rs1695 G, and *NACC2* rs4409473 C, which have been already described above.

4.4.1 *ACE* rs4646994 I allele

Circulating angiotensin I-converting enzyme (ACE) exerts a tonic regulatory function in circulatory homeostasis, through the synthesis of vasoconstrictor angiotensin II, which also drives aldosterone synthesis, and the degradation of vasodilator kinins. A polymorphism in intron 16 of the human *ACE* gene (location: 17q23.3) has been identified in which the presence (insertion, I allele) rather than the absence (deletion, D allele) of a 287-bp Alu-sequence insertion fragment is associated with lower serum and tissue ACE activity. An excess of the I allele has been associated with some aspects of endurance performance, being identified in 25 elite mountaineers (Montgomery et al., 1998) and 34 elite British ≥5000 m distance runners (Myerson et al., 1999). In addition, an excess of the I allele is present in elite Australian ($n=64$) (Gayagay et al., 1998), Croatian ($n=40$) (Jelakovic et al., 2000), and Russian ($n=107$) (Ahmetov et al., 2008) rowers as well as Spanish elite athletes (25 cyclists, 20 long-distance runners, and 15 handball players)

(Alvarez et al., 2000). *ACE* I allele is also over-represented among 100 fastest Ironman triathletes (Collins et al., 2004), 27 elite Spanish runners (Lucia et al., 2005a), successful marathon runners (scoring on places from 1st to 150th) (Hruskovicová et al., 2006), 35 outstanding Russian middle-distance athletes (24 swimmers, 7 track-and-field endurance athletes, and 4 cross-country skiers) (Nazarov et al., 2001), 33 Italian Olympic endurance athletes (10 road cyclists, 7 track-and-field runners, and 16 cross-country skiers) (Scanavini et al., 2002), 80 Turkish endurance and power/endurance athletes (17 middle-distance running, 10 basketball, 18 handball, and 35 football players) (Turgut et al., 2004), 16 long-distance (25 km) swimmers from different nationalities (Tsianos et al., 2004), 55 elite Polish rowers (Cieszczyk et al., 2009), 108 Japanese university long-distance runners (Min et al., 2009), and 29 Indian Army triathletes (Shenoy et al., 2010).

It should be noted that several studies have demonstrated no association between the *ACE* I/D polymorphism and endurance athlete status (Ash et al., 2011; Ahmetov et al., 2009b; Papadimitriou et al., 2009; Orysiak et al., 2013; Rankinen et al., 2000; Scott et al., 2005; Taylor et al., 1999; Tobina et al., 2010) or prevalence of the D allele (or low proportion of the II genotype) in endurance-oriented athletes in comparison with controls (Amir et al., 2007; Ginevičienė et al., 2011; Lucia et al., 2005a; Muniesa et al., 2010). Furthermore, Tobina et al. (2010) have shown that average running speed was significantly higher for those Japanese endurance runners with the combined DD/ID genotypes than for those with the II genotype.

4.4.2 *ACTN3* rs1815739 577X allele

The α-actinins constitute the predominant protein component of the sarcomeric Z line in skeletal muscle fibers, where they form a lattice structure that anchors together actin-containing thin filaments and stabilizes the muscle contractile apparatus. Expression of the α-actininin-3 (ACTN3) is limited to fast muscle fibers responsible for generating force at high velocity. A common genetic variation in the *ACTN3* gene (location: 11q13.1) that results in the replacement of an arginine (Arg or R) with a stop codon at amino acid 577 (C-to-T transition in exon 16; rs1815739; R577X) had been identified. The 577X allele contains a sequence change that completely prevents the production of functional α-actinin-3 protein. The loss of α-actinin-3 expression in a knockout mouse model results in a shift in muscle metabolism toward the more efficient aerobic pathway and an increase in intrinsic endurance performance (MacArthur et al., 2007). Although four studies have shown that proportion of the XX genotype and/or X allele was higher in endurance-oriented athletes compared with controls (Eynon et al., 2009b; Shang et al., 2010; Yang et al., 2003; Joanna et al., 2015), the majority of authors reported no association between the *ACTN3* R577X polymorphism and endurance athlete status (Döring et al., 2010; Ginevičienė et al., 2011; Grealy et al.,

2013; Lucia et al., 2006; Mikami et al., 2014; Niemi and Majamaa, 2005; Papadimitriou et al., 2008; Paparini et al., 2007; Saunders et al., 2007; Tsianos et al., 2010; Wang et al., 2013b; Yang et al., 2007; Papadimitriou et al., 2018) or even an inverse correlation (i.e., over-representation of the RR + RX genotypes in endurance athletes) in different cohorts (Ahmetov et al., 2010; Kikuchi et al., 2016).

4.4.3 *ADRB2* rs1042713 16Arg allele

The β-2 adrenergic receptor (encoded by *ADRB2*; location: 5q31-q32) is a member of the G protein-coupled receptor superfamily, expressed in many cell types throughout the body and plays a pivotal role in the regulation of the cardiac, pulmonary, vascular, endocrine, and central nervous system. Kochanska-Dziurowicz et al. (2013) have reported that the performed work in the maximal incremental exercise test of regularly training young ice hockey players depended on the initial levels of noradrenaline in plasma and *ADRB2* mRNA in peripheral blood mononuclear cells. The Gly16Arg SNP (rs1042713 G/A) of the *ADRB2* gene and its association with several phenotypes has been described. Specifically, the 16Arg allele was associated with lower receptor density and resting cardiac output (Snyder et al., 2006). Wolfarth et al. (2007) reported that the 16Arg allele was over-represented in 313 white male elite endurance athletes compared to 297 white male sedentary controls, suggesting a positive association between the tested Gly16Arg polymorphism and endurance performance. Furthermore, in a study of 316 Mount Olympus marathon runners, Tsianos et al. (2010) have shown an association between the 16Arg allele and the fastest time of athletes. The results of these studies are in agreement with the previous work in which an association of the 16Arg allele with higher peak VO_2 in heart failure patients was reported (Wagoner et al., 2000). On the other hand, Sawczuk et al. (2013a) found no differences in allelic frequencies of this polymorphism between 123 Polish endurance athletes and controls.

4.4.4 *AQP1* rs1049305 C allele

Aquaporins are a family of small integral membrane proteins related to the major intrinsic protein (MIP or AQP0). The aquaporin-1 (AQP1) is the best known and most studied of this family. *AQP1* gene (location: 7p14) encodes for a protein responsible for transporting large amounts of water across cell membranes. AQP1 has been identified in various tissues, including red blood cells, endothelial cells, as well as smooth, skeletal and cardiac muscle. During osmotic stress, such as occurs during intense exercise, AQP1 facilitates the transfer of water from the blood into the muscle, provides osmotic protection, and promotes water reabsorption. Martínez et al. (2009) have examined the association between *AQP1* gene rs1049305 C/G polymorphism (in the 3′ untranslated region) and athletic performance in 784 Hispanic international level marathon runners. Athletes were divided into two groups: (1) cases ($n = 396$), finished in the top third tertile

for their age and gender and (2) controls ($n=388$), finished in the lowest third tertile. The frequency of the rare C allele was significantly higher in cases than in controls (36.0% vs 30.0%; $P=0.005$). In a following study of 91 international 10 km runners, the same group of authors have demonstrated that carriers of the *AQP1* rs1049305 C allele had a significantly greater body fluid loss (3.7 ± 0.9 kg) than noncarriers (1.5 ± 1.1 kg; $P<0.05$) (Rivera et al., 2011). In a recent study of 504 Ironman triathletes, Saunders et al. (2014) found that triathletes who carry a C allele completed the 42.2-km run stage faster than triathletes with a GG genotype ($P=0.032$), confirming that *AQP1* rs1049305 C allele may be favorable for endurance performance.

4.4.5 *AMPD1* rs17602729 Gln12 allele

Adenosine monophosphate deaminase 1 (AMPD1) catalyzes the deamination of adenosine monophosphate to inosine monophosphate in skeletal muscle. Deficiency of the AMPD1 is apparently a common cause of exercise-induced myopathy and probably the most common cause of metabolic myopathy in the human. In the overwhelming majority of cases, AMPD1 deficiency is due to a 34 C/T transition in exon 2 (rs17602729 C/T) of the *AMPD1* gene (location: 1p13), which creates a nonsense codon (Gln12X) that prematurely terminates translation. In a study of Rico-Sanz et al. (2003), subjects with the *AMPD1* XX genotype had diminished exercise capacity and cardiorespiratory responses to exercise in the sedentary state. In a study of 935 Coronary artery disease patients, the carriers of the X allele had a significantly lower relative increase in peak VO_2 after 3 months of aerobic training (Thomaes et al., 2011). Finally, two studies reported low frequency of the mutant X allele in a group of top-level Spanish male endurance athletes (cyclists and runners, $n=104$) (Rubio et al., 2005) and 127 Polish rowers (Cięszczyk et al., 2011) compared with controls. However, this observation was not confirmed by Ginevičienė et al. (2014), when 84 Lithuanian athletes were compared with 260 controls.

4.4.6 *BDKRB2* −9 allele

Bradykinin is a potent endothelium-dependent vasodilator and acts via the bradykinin B2 receptor (encoded by *BDKRB2*; location: 14q32.1-q32.2). The absence (−9), rather than the presence (+9), of a 9-bp repeat sequence in exon 1 has previously been shown to be associated with increased gene transcription and higher *BDKRB2* mRNA expression. Williams et al. (2004) have shown that the −9 allele of the *BDKRB2* gene is associated with higher efficiency of muscular contraction (i.e., the energy used per unit of power output during exercise or delta efficiency). In 81 elite British runners, analysis revealed a linear trend of increasing −9 allele frequency with distance run. The proportion of −9 alleles increased from 0.382 to 0.412 to 0.569 for those athletes running ≤200 m, 400–3000 m, and ≥5000 m, respectively (Williams et al., 2004). The −9/−9 genotype

of the *BDKRB2* gene was also over-represented in male Caucasian triathletes ($n=443$) of the 2000 and 2001 South African Ironman Triathlons compared to male controls ($n=203$) (Saunders et al., 2006). In addition, when divided into tertiles according to their finishing times, the −9/−9 genotype was only over-represented in the fastest tertile. However, Eynon et al. (2011) found no significant differences in the frequencies of the −9 allele and −9/−9 genotype between 74 Israeli endurance athletes and 240 controls. The same negative results were obtained by Sawczuk et al. (2013b) and Grenda et al. (2014) in two studies involving 334 endurance athletes from Russia and Poland.

4.4.7 *COL5A1* rs12722 T allele

Collagens are a group of extracellular matrix proteins, and are the most abundant proteins in mammals, making up about 25%–35% of the whole-body protein content. Collagens, in the form of elongated fibrils, are mostly found in connective (fibrous) tissues such as tendon, ligament, and skin, and are also abundant in cornea, cartilage, bone, blood vessels, the gut, and intervertebral disc. The *COL5A1* gene (location: 9q34.2-q34.3) encodes the pro-α1 chain of type V collagen, the rate-limiting component of the of type V collagen trimer assembly. The *COL5A1* gene rs12722 C/T polymorphism has recently been shown to be associated with passive straight leg raise and/or a sit-and-reach measurement (the carriers of the rs12722 T allele were more inflexible) (Brown et al., 2011a; Collins et al., 2009). Since inflexibility improves running performance, possibly through enhancing the storage and return of energy and minimizing the need for muscle-stabilizing activity, it was hypothesized that the rs12722 T allele would associate with improved running performance. Indeed, in a study of 313 Caucasian Ironman triathletes, Posthumus et al. (2011) have shown that participants with a TT genotype completed the running component (42.2 km) of the race significantly faster than individuals with a CC genotype. These results were then replicated in a second association study with 72 ultra-marathon runners (56 km): participants with a TT genotype completed the ultramarathon significantly faster than participants with TC and CC genotypes. Furthermore, when the cohort was divided into performance and flexibility quadrants, the rs12722 T allele was significantly over-represented within the fast and inflexible quadrant (Brown et al., 2011b).

4.4.8 *GABPB1* rs12594956 A and rs7181866 G alleles

The GA-binding protein transcription factor, β subunit 1 (GABPB1; also known as NRF2) protein is a transcriptional regulator of genes involved in activation of cytochrome oxidase expression and nuclear control of mitochondrial function. Increase in *NRF2* represents key regulatory component of the stimulation of mitochondrial biogenesis by exercise. Mitochondrial transcription factor A (TFAM), cytochrome *c,* and heme biosynthesis proteins are regulated by NRF2.

It was shown that polymorphisms of the *GABPB1* gene (location: 15q21.2) may explain variance in endurance capacity and affect elite endurance performance. More specifically, He et al. (2007) examined the association between the *GABPB1* genotypes and endurance capacity (running economy and VO_{2max}) measured prior to and after endurance training program in young Chinese men. At baseline, there was an association between the VO_{2max} and *GABPB1* rs12594956 A/C polymorphism. Training response of VO_2 at running economy was associated with *GABPB1* rs12594956 A/C and rs7181866 A/G polymorphisms.

In two studies involving 155 Israeli athletes and 240 nonathletes, Eynon et al. (2009d, 2010b) have analyzed the distribution of two *GABPB1* SNPs (rs12594956 A/C and rs7181866 A/G). The frequencies of the rs12594956 AA and rs7181866 AG genotypes were significantly higher in endurance-oriented athletes ($n=74$) than in sprinters ($n=81$) or controls. In a following study, Eynon et al. (2013) have shown that the frequency of the AA genotype of the rs12594956 A/C polymorphism was significantly higher in 89 Spanish world-class endurance athletes compared with 38 power athletes ($P<0.01$) and 110 controls ($P<0.01$) (48% vs 13% and 21%, respectively). However, the frequencies of the rs7181866 polymorphisms did not differ between endurance athletes and controls. Furthermore, Maciejewska-Karlowska et al. (2012) confirmed the association between the rs7181866 A/G polymorphism and endurance athlete status, that is the proportion of the AG genotype and the frequency of the G allele were significantly higher in 55 Polish male rowers in comparison with 130 controls.

4.4.9 *HFE* rs1799945 63Asp allele

Hereditary hemochromatosis (HFE) is an autosomal recessive disease in which the body's iron stores are increased. The HFE gene (location: 6p21.3) plays a major role in hereditary HFE. The HFE protein functions to regulate iron absorption by regulating the interaction of the transferrin receptor with transferrin. Most patients with the manifest of hereditary HFE are homozygous for the Cys282Tyr mutation, and a small proportion are heterozygous for both the Cys282Tyr and His63Asp (rs1799945 C/G or H63D) mutation of the *HFE* gene. The *HFE* gene His63Asp polymorphism was shown to be associated with blood iron indices (subjects with one or more mutations show higher blood iron concentrations and transferrin saturation than subjects without mutations) (Burt et al., 1998). Interestingly, Deugnier et al. (2002) have shown an increased frequency of the 63Asp allele in 83 elite French road male cyclists when compared to controls ($P=0.04$). In the other research, Asp/Asp + His/Asp genotypes were higher in the group of French elite athletes ($n=129$) compared to controls ($n=219$, 38% vs 21.9%, $P=0.0019$) (Hermine et al., 2015). Consistently with these findings, in a third study of 65 elite Spanish endurance-oriented athletes (50 professional road cyclists and 15 Olympic class endurance runners), Chicharro et al. (2004) have found that the

frequency of the His/Asp genotype was significantly higher in athletes in comparison with 134 controls (41.5% vs 24.6%; $P=0.01$), suggesting that 63Asp allele may confer some advantage in endurance performance.

4.4.10 *KCNJ11* rs5219 Glu23 allele

Potassium channels are present in most mammalian cells, where they participate in a wide range of physiologic responses. The potassium in wardly rectifying channel, subfamily J, member 11 (encoded by *KCNJ11*; location: 11p15.1) is an integral membrane protein and inward rectifier-type potassium channel. The encoded protein, which has a greater tendency to allow potassium to flow into a cell rather than out of a cell, is controlled by G-proteins. The *KCNJ11* gene is expressed in several tissues, including cardiac and skeletal muscle, where it is involved in the coupling of cell metabolism to cell electrical activity. Among several potentially functional genetic variants identified in the *KCNJ11* gene, the Glu23Lys (E23K or rs5219 C/T) variant has been the most extensively studied and has been found to be associated with various glucose, insulin and cardiovascular phenotypes and type 2 diabetes risk. Yi et al. (2008) have shown that the Glu/Glu genotype was associated with the highest values of $\dot{V}O_{2max}$ and maximal minute ventilation in women in untrained state than in Glu/Lys heterozygotes. Furthermore, two independent case-control studies have demonstrated that the *KCNJ11* Glu23 is significantly over-represented in endurance-oriented athletes compared to controls in mixed Caucasian (184 male endurance-oriented athletes with $\dot{V}O_{2max} \geq 75$ mL/kg/min; 61.0% vs 50.0%, $P=0.01$) (González et al., 2003) and Spanish (98 marathon runners; 68.0% vs 53.0%, $P=0.04$) (Ortiz et al., 2005) cohorts.

4.4.11 *MCT1* rs1049434 *Glu490 allele*

During exercise, lactate is transported across the plasma membrane via a cell-cell lactate shuttle, which is facilitated by membrane-bound proton-linked monocarboxylate transporters (MCTs) that are pH dependent. MCT1 [encoded by *MCT1* (also known as *SLC16A1*); location: 1p12] has been found predominantly in oxidative muscle fibers and is required for lactate produced by white muscle fibers to enter the myocytes for oxidation in heart and red skeletal muscle that use lactate as a major respiratory fuel. Merezhinskaya et al. (2000) were the first to describe the common missense mutation A1470T (rs1049434) in the *MCT1* gene, which leads to change in codon 490 of glutamic acid to aspartic acid (Glu490Asp). Individuals with mutant T allele had lactate transport rates 60%–65% lesser of mean normal. Recently, Cupeiro et al. (2010) have examined the influence of the *MCT1* Glu490Asp polymorphism on lactate accumulation after training. Male carriers of the *MCT1* 490Asp (T) allele showed higher lactate accumulations than noncarriers during circuit weight training. This observation was confirmed in 79 Russian rowers during an incremental test to exhaustion on a rowing ergometer (Fedotovskaya et al., 2014).

Furthermore, Fedotovskaya et al. (2014) found that the frequencies of the Glu490 (A) allele (71.8% vs 62.5%, $P<0.0001$) and Glu/Glu genotype (59.8% vs 39.4%, $P<0.0001$) were significantly higher in Russian endurance-oriented athletes ($n=142$) compared with the control group ($n=467$), indicating that Glu490 allele (lower lactate accumulation variant) is favorable for endurance performance. This observation (over-representation of the Glu/Glu genotype) was confirmed when top Israeli long-distance runners of Ethiopian origin ($n=37$) were compared to controls (Ben-Zaken et al., 2019). However, these results were not replicated in Polish and Israeli (of non-Ethiopian origin) endurance-oriented athletes (Sawczuk et al., 2015; Ben-Zaken et al., 2015).

4.4.12 MtDNA H haplogroup and K haplogroup (unfavorable)

Mitochondria are essential to all higher organisms for sustaining life, and are extremely important in energy metabolism, providing 36 molecules of adenosine triphosphate (ATP) per glucose molecule in contrast to the two ATP molecules produced by glycolysis. Although most DNA is packaged in chromosomes within the nucleus, mitochondria also possess their own circular DNA: mtDNA. The 16569-bp human mtDNA contains 13 genes for mitochondrial oxidative phosphorylation (OXPHOS), as well as two ribosomal RNA and 22 transfer RNA genes that are necessary for protein synthesis within mitochondria. Patients with mutations in mtDNA commonly present with exercise intolerance, muscle weakness, and increased production of lactic acid. Some studies reported association between the mtDNA polymorphism and athlete status. In a study of Finnish elite endurance athletes ($n=52$), an excess of mtDNA haplogroup H and the absence of haplogroup K compared to 1060 controls and 89 sprinters was reported (Niemi and Majamaa, 2005). Maruszak et al. (2014) have also shown that haplogroup K was less prevalent in 130 male Polish endurance athletes than in 413 controls, while haplogroup H was over-represented in elite endurance athletes compared with elite power athletes or controls.

4.4.13 *PPARA* rs4253778 G allele

Peroxisome proliferator-activated receptor α (PPARα) is a transcription factor that regulates lipid, glucose, and energy homeostasis and controls body weight and vascular inflammation. PPARα is expressed at high levels in tissues that catabolize fatty acids, notably liver, skeletal muscle, and heart, and at lower levels in other tissues, including pancreas. The level of expression of PPARα is higher in type I (slow-twitch) than in type II (fast-twitch) muscle fibers. PPARα regulates the expression of genes encoding several key muscle enzymes involved in fatty acid oxidation.

Exercise-induced left ventricular (LV) growth in healthy young men was strongly associated with the intron 7 G/C (rs4253778) polymorphism of the *PPARA* gene (location: 22q13.31) (Jamshidi et al., 2002). Individuals homozygous for the C allele had a

threefold greater and heterozygotes had twofold greater increase in LV mass than G allele homozygotes. It was demonstrated that the frequency of the *PPARA* rs4253778 GG genotype and G allele was higher in 491 Russian endurance-oriented athletes ($P=0.0001$) (Ahmetov et al., 2006), 74 elite Israeli endurance athletes ($P=0.051$) (Eynon et al., 2010a), 55 elite Polish rowers ($P=0.009$) (Maciejewska et al., 2011), Polish combat athletes ($P=0.01$) (Cieszczyk et al., 2011), and 60 Turkish endurance athletes ($P<0.001$) (Tural et al., 2014) compared to controls and/or sprinters. In accordance with the hypothesis, mean percentage of type I muscle fiber was higher in GG homozygotes than in CC genotype subjects (in a study of 40 physically active healthy men) (Ahmetov et al., 2006). Furthermore, GG genotype was shown to be correlated with high values of oxygen pulse both in male and female Russian rowers (Akhmetov et al., 2007).

4.4.14 *PPARD* rs2016520 C allele

Peroxisome proliferator-activated receptor δ (PPARδ) is a transcription factor involved in regulation of genes implicated in fatty acid oxidation, cholesterol metabolism, and thermogenesis. Overexpression of a constitutively active PPARδ (VP16-PPARδ) in skeletal muscles of transgenic mice preprograms an increase in oxidative muscle fibers, enhancing running endurance by nearly 100% in untrained adult mice (Wang et al., 2004). PPARδ agonist GW1516 (experimental drug that has been used in the treatment of obesity, metabolic syndrome, and type 2 diabetes) may increase the exercise tolerance and therefore, is included in the WADA prohibition list (Brzeziańska et al., 2014). The SNP located at the 5′-UTR region of the exon 4 (rs2016520, referred as +294 T/C or +15 C/T or c.−87T/C) variant in *PPARD* gene (location: 6p21.2) has been intensively studied. Skogsberg et al. (2003) have shown that the rare C allele had higher transcriptional activity than the common T allele. Furthermore, the *PPARD* C allele has been reported to be significantly associated with an increased muscle glucose uptake (Vanttinen et al., 2005). In addition, a significantly higher frequency of the *PPARD* C allele was observed in long endurance ($n=308$, 19%), middle endurance ($n=220$, 17.5%), and short endurance ($n=81$, 20.4%) Russian athletes compared to controls ($n=610$, 12.1%) (Ahmetov et al., 2007). Furthermore, in a study of 155 Israeli athletes, Eynon et al. (2009c) have found that the frequency of the combination *PPARD* CC + *PPARGC1A* Gly/Gly was significantly higher in elite endurance-oriented athletes compared with non-elite athletes. However, contrary to the hypothesis that *PPARD* C allele may be advantageous for the endurance performance, Hautala et al. (2007) in considering only black ($n=264$) subjects, have demonstrated in *PPARD* CC homozygotes a smaller endurance training-induced increase in maximal oxygen consumption and maximal power output compared to T allele carriers. Maciejewska-Karlowska et al. (2014) also found inverse association between the C allele and endurance athlete status by studying 120 Polish endurance athletes.

4.4.15 *PPARGC1A* rs8192678 Gly482 allele

Peroxisome proliferator-activated receptor γ (PPARγ) coactivator 1α (PGC1α, encoded by *PPARGC1A*), a transcriptional coactivator of PPAR family, is involved in mitochondrial biogenesis, fatty acid oxidation, glucose utilization, thermogenesis, angiogenesis, and muscle fiber-type conversion toward slow-twitch type I fibers. The minor serine-encoding allele of the common Gly482Ser polymorphism (rs8192678 G/A) in *PPARGC1A* gene (location: 4p15.1) is associated with reduced expression of *PPARGC1A* (Ling et al., 2004). Furthermore, the 482Ser allele has been reported to be associated with less increase in individual anaerobic threshold after 9 months of aerobic training (Stefan et al., 2007), lower aerobic capacity in Russian rowers (Akhmetov et al., 2007) and mixed group of Spanish endurance athletes, fit, and unfit Caucasian controls (Lucia et al., 2005b). In addition, in four case-control studies, significantly lower frequency of 482Ser allele in Spanish ($n=104$), Russian ($n=579$), Israeli ($n=74$), and Polish ($n=92$) elite endurance-oriented athletes has been reported (Lucia et al., 2005b; Ahmetov et al., 2009b; Eynon et al., 2010a; Maciejewska et al., 2012). However, while Maruszak et al. (2014) and He et al. (2015) have not replicated the same results in 213 Polish and 235 Chinese endurance athletes, respectively, Tural et al. (2014) have shown an opposite association, that is 60 Turkish endurance athletes had significantly lower frequency of the Gly482 allele than 110 controls.

4.4.16 *VEGFR2* rs1870377 472Gln allele

Vascular endothelial growth factor (VEGF) is a major growth factor for endothelial cells and VEGF receptor 2 [VEGFR2; also known as kinase insert domain receptor (KDR)] is essential to induce the full spectrum of VEGF angiogenic responses to aerobic training. One of the potential functional polymorphisms of the *VEGFR2* gene (location: 4q11-q12) is the rs1870377 T/A variant, which determines a histidine (His) to glutamine (Gln) substitution. Studies have reported that the His472Gln polymorphism influences the efficiency of VEGF binding to VEGFR2 (Wang et al., 2007). In a study of 182 endurance-oriented Russian athletes, significantly higher frequency of the *VEGFR2* 472Gln allele compared to controls was reported (Ahmetov et al., 2009a). Furthermore, the 472Gln allele was also shown to be significantly associated with a higher proportion of type I fibers of *M. vastus lateralis* (determined by immunohistochemistry) in both athletes (all-round speed skaters, $n=23$; age 20.4 ± 0.5 years) and physically active men ($n=45$; age 23.5 ± 0.4 years), and with a greater VO_{2max} in female rowers (Ahmetov et al., 2009a). Eider et al. (2013) consistently with these observations have found that the frequencies of the *VEGFR2* Gln/Gln genotype (21.02% vs 10.56%; $P=0.014$) and 472Gln allele (41.19% vs 29.50%; $P=0.001$) were significantly higher in endurance-orientated athletes ($n=176$) compared to sedentary controls ($n=161$).

4.4.17 *UCP2* rs660339 55Val allele

The uncoupling proteins 1, 2, and 3 (UCP1, UCP2, and UCP3) are members of the superfamily of anion carrier proteins located in the inner membrane of mitochondria. The UCP2 protein (encoded by *UCP2*) is involved in uncoupling OXPHOS from ATP synthesis in certain tissues and regulation of lipid metabolism and energy expenditure. Endurance training leads to an increase in UCP2 mRNA and protein content in skeletal muscles, pancreatic islets, and heart. A common Ala55Val polymorphism (rs660339 C/T) has been described in the *UCP2* gene (location: 11q13) and has been variably associated with altered body mass index, physical activity, and changes in energy expenditure. More specifically, the Val/Val genotype has been reported to be associated with higher exercise efficiency (Buemann et al., 2001), enhanced metabolic efficiency and physical activity (Astrup et al., 1999), and higher VO_{2max} in 27 male Russian rowers (Ahmetov et al., 2008). Recently, it has been shown that the frequency of the 55Val allele is over-represented in 694 Russian elite endurance athletes (Ahmetov et al., 2009b) compared to 1132 controls, and in the group of more successful Polish runners (<100 min, $n=76$) compared to the >100 min Polish group ($n=104$, $OR=4.23$, $P<0.0001$) (Gronek et al., 2018).

4.4.18 *UCP3* rs1800849 T allele

The expression of *UCP3* mainly in skeletal muscle mitochondria made UCP3 an attractive target for studies toward manipulation of energy expenditure to fight disorders such as obesity and type 2 diabetes. In humans, acute exercise induces upregulation of UCP3, most likely because of elevated plasma free fatty acid levels (Schrauwen and Hesselink, 2002). Several polymorphisms in the *UCP3* gene (location: 11q13.4) have been identified and related to markers of energy metabolism and aerobic capacity (Ahmetov et al., 2008; Schrauwen and Hesselink, 2002). One of the early detected observations was 5′UTR −55 C/T polymorphism (rs1800849), of which the T allele was reported to be associated with increased skeletal muscle UCP3 mRNA expression (Schrauwen et al., 1999), and increased aerobic capacity in Russian female rowers (Ahmetov et al., 2008). The frequency of the *UCP3* T allele was significantly higher in 694 Russian elite endurance athletes compared to 1132 controls (Ahmetov et al., 2009b). In a Genathlete study, the difference in *UCP3* TT genotype frequency between 183 endurance athletes and 121 controls almost reached significance level (12.0% vs 6.0%; $P=0.076$) (Echegaray et al., 2003). However, Hudson et al. (2004) have found no association between the −55 C/T polymorphism within the *UCP3* gene and the ultra-endurance performance of triathletes who completed either the 2000 or 2001 South African Ironman triathlons.

4.5 Conclusion

The current review provides evidence that at least 100 genetic markers are linked to elite endurance athlete status. However, it should be emphasized that most (78%) of the case-control and association studies have not yet been replicated in independent samples. Based on that, we strongly believe that even more research is needed before these findings can be extended to practice in sport. On the other hand, since sport-related DNA polymorphisms do not fully explain the heritability of athlete status, other forms of variation, such as rare mutations and epigenetics markers (i.e., stable and heritable changes in gene expression), must be considered. The issues with respect to appropriate study designs, sample size, population stratification, and quality of the genotype/phenotype measurement are also of great importance.

The impact of genetics in sports and exercise appears to have multiple influences. Its positive effect on exercise performance must be combined with effective training programs and favorable lifestyle habits for success in sports and health benefits (Pokrywka et al., 2013). Accordingly, one of the applications of sports genetics could be the development of predictive genetic performance tests, although it is still too premature currently in sports genomics to be able to definitively test for predictive genetic markers (Webborn et al., 2015). Furthermore, the application of genetic testing in sports could provide new opportunities for sports clubs to understand athletes' susceptibility for certain pathological states (injuries, cardiomyopathies, sudden death, etc.), map genetic suitability for specific team positions and roles, and to gain insights into athletes' development in various sports or physical activities. Future research including multicenter GWASs and whole-genome sequencing in large cohorts of athletes with further validation and replication will substantially contribute to the discovery of large number of the causal genetic variants (mutations and DNA polymorphisms) that would partly explain the heritability of endurance athlete status and related phenotypes.

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CHAPTER FIVE

Genetics of team sports

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5.1 Introduction

Athletic performance is one of the most complex human phenotypic traits influenced by anthropometric (Massidda et al., 2013), psychological (Peplonska et al., 2017), and physiological properties (Sandbakk and Holmberg, 2017), as well as by the training, nutrition, and health status of the individual athlete (Eynon et al., 2012a). It is assumed, however, that athletic performance variations among athletes are partly due to small contributions of hundreds of genes (Pitsiladis et al., 2013; Cięszczyk et al., 2016b). To date, most of the exercise genomics studies have focused on genotyping predominantly sprint/power or endurance athletes, who represent the physiological extremes of the sporting continuum.

However, the genetic contribution to success in sports that require a mixture of anaerobic and aerobic qualities has received limited attention (Eynon et al., 2014; Massidda et al., 2015b; Cięszczyk et al., 2016a). Moreover, perhaps because of the insufficient sample size associated with the low number of elite athletes available for analysis, most studies on the genetics of team sports have been conducted on the team as a whole, producing contrasting results (Egorova et al., 2014; Ginevičiene et al., 2014; Massidda et al., 2015b; Cięszczyk et al., 2016a).

The present chapter provides a background on the studies currently performed in the field of genetics of team sports. Starting with a brief description of the physiological demands of team sports performance, this chapter illustrates the candidate genetic variants thought likely to influence the probability of becoming an elite team sports athlete and excel in the key phenotypes that are relevant to team sports performance. Particular emphasis will be placed on football and rugby, the two team sports better studied in the field of genomics.

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5.2 Phenotype description of team sports performance

Team sports represent a good illustration of the relationship between teamwork and sports in which different players interact directly and simultaneously to achieve a shared objective with the goal of winning. The "traditional" team sports are often referred to as 'invasion sports' and practiced between two opposite teams with the aim to facilitating the movement of a ball or similar object (such as the vulcanized rubber disk in ice hockey), often to a scoring area, according to a set of rules and in order to score points.

The members of the team, generally more than three and with different roles, make decisions, communicate, manage conflict, and solve problems. They often combine those psychological and tactical skills with physical dominance over opposition team members in terms of speed, agility, strength, power, and endurance. The main "traditional" team sports are represented by American Football, Australian Rules Football, Baseball, Basketball, Beach Volleyball, Cricket, Curling, Field Hockey, Futsal, Football (Soccer), Gaelic Football, Handball, Ice Hockey, Lacrosse, Rugby Union (RU), Rugby League, Rugby Sevens, Softball, Volleyball, and Water Polo.

In recent years, the meaning of a "team sport" has been disputed. In fact, some types of sports often considered as team sports involve less than three players per team or have different objectives or rules than "traditional" team sports. Some examples include Bobsleigh, Cycling, Dragon Boat Racing, Rhythmic Gymnastics, Rowing, Sailing, Swimming (artistic), Tennis (doubles), Table Tennis, and Track and Field (relays). Seven team sports are currently on the program of the Summer Olympics (Basketball, Football, Field Hockey, Handball, Rugby Sevens, Volleyball, and Water Polo) with competition for both men and women. Curling and Ice Hockey are team sports at the Winter Olympics together with the Bobsleigh competition where the men's event has classes for both two-man and four-man sleds, but the women's class is restricted to two persons only.

Athletes engaged in "traditional" team sports are required to repeatedly produce complex motor skills (e.g., passing, defending, and tackling) and maximal or submaximal efforts (e.g., accelerations, changes in pace and direction, sprints, jumps, and kicks) interspersed with brief active recovery, with and without the ball/puck, over an extended period of time (1–2 h) (Bishop and Girard, 2013). Due to its acyclical nature and intensity, "traditional" team-sports can be classified as a high-intensity intermittent activity in which fatigue manifests as a reduced ability to produce maximal force or power during a competitive match and is reflected in a decline in performance (Reilly, 1994a; Waldron and Highton, 2014). Although the predominant metabolic pathway of the team sports is ultimately aerobic, high functioning anaerobic energy systems are essential to the outcome of a team sports match because they strongly influence performance during the key phases of a match (Rampinini et al., 2007).

Physical demands vary widely between different team sports. Certain multidirectional sports require frequent and consistent sagittal plane sprinting and high-intensity running (Gabbett et al., 2008; Bradley et al., 2011), while others demand more lateral shuffling or cutting, and some are heavily reliant on jumping (Matthew and Delextrat, 2009; Scanlan et al., 2011; Póvoas et al., 2014). Many team sports (e.g., Football, RU, Rugby League, and Australian Football) require the ability to sustain high-intensity efforts, interspersed with longer intervals of submaximal exercise (Mohr et al., 2005). The ability to rapidly accelerate and decelerate (even without reaching a high running speed), sprinting and jumping performance, and repeated sprint ability (RSA) are considered important key factors for team sports performance (Little and Williams, 2005). The physical demands are therefore complex, requiring athletes to have highly developed speed, agility, muscular strength and power, and endurance.

During competitive field-based team sports, such as Football, Field Hockey, RU, Rugby League, and Australian Football, elite athletes may cover 4–14 km at an average intensity of ~85%–90% of their maximal heart rate (HR_{max}) or 75%–80% of their maximal rate of oxygen uptake (VO_{2max}), with marked differences according to playing position (Gabbett, 2005; Spencer et al., 2005; Bangsbo et al., 2006; Aughey, 2011; Jones et al., 2015). This suggests that, together with anaerobic performance capabilities, a well-developed aerobic energy system is an important physiological determinant of team sport physical performance.

In conclusion, there are many physiological qualities important for team sport performance that must be considered in genetic research. Considerable variability exists in the demand for straight-line running, lateral movement, cutting and jumping across sports, ages, and positional roles. This phenotype heterogeneity means that genomic studies should include an appropriate sample selection based on the unique physiological demands of each sport and of the positional roles within them, rather than including multiple team sports in the same analysis.

5.3 Genetic markers associated with athletic status and key phenotypes in team sports

The genome of team sports athletes has been recently investigated to evaluate potential associations of certain genetic variants with sports performance. Although several studies have been performed to investigate the genetic variants associated with the elite athletic performance, the genetic data on team sports athletes remain extremely limited. So far, only 10 genetic markers associated with team sports athlete status have been identified (Table 5.1) and replication studies are needed to verify those. One of the main problems that emerge from the literature is the heterogeneity of the studies with regard to the classification of team sports. In fact, for example, some studies combine athletes from sports like volleyball with samples of athletes who compete in individual

Table 5.1 Gene variants (genetic markers) for team sport athlete status

Gene	Location	Polymorphism	Favorable allele	Team sport (position)
ACE	17q23.3	Alu I/D (rs4646994)	D	Football (all, goalkeepers)
ACTN3	11q13.1	Arg577Ter (rs1815739 C/T)	C (Arg577)	Football (all, forwards) Rugby (back three)
AMPD1	1p13	Gln12Ter (rs17602729 C/T)	C (Gln12)	Football (all)
COL5A1	9q34.2-q34.3	rs12722 C/T	C	Rugby (all)
		rs3196378 C/A	C	Rugby (all; back three and centers)
FTO	16q12.2	rs9939609 A/T	T	Rugby (back three and centers)
MCT1	1p12	Glu490Asp (rs1049434 A/T)	A (Glu490)	Football (forwards) Volleyball (all)
NOS3	7q36	rs2070744 T/C	C	Football (all)
PPARA	22q13.31	rs4253778 G/C	C	Football (all, forwards)
UCP2	11q13	Ala55Val (rs660339 C/T)	T (55Val)	Football (all, defenders, mid-fielders)

sprint/power disciplines (Sessa et al., 2011; Ruiz et al., 2013; Eynon et al., 2014), while others use "mixed sports" samples in which there are both team and individual sports represented (Orysiak et al., 2014, 2015; Durmic et al., 2017).

Currently, only three genetic studies have focused specifically on team sports, which include more than one sport. The first study on a group of team sports athletes was performed by Ahmetov et al. (2013), which analyzed the distribution of peroxisome proliferator-activated receptor alpha (*PPARA*) genotypes in 665 male and female Russian athletes. The sample comprised regional and national competitive standard athletes from the following sporting disciplines: badminton ($n=16$), baseball ($n=28$), basketball ($n=85$), beach volleyball ($n=10$), court tennis ($n=33$), football ($n=241$), futsal ($n=9$), handball ($n=24$), ice hockey ($n=55$), rugby ($n=48$), softball ($n=31$), table tennis ($n=14$), volleyball ($n=53$), and water polo ($n=18$). The frequency of the *PPARA* C allele was significantly higher in athletes compared to 1706 healthy unrelated (males and females) controls (20.5% vs 16.4%, $P=0.0009$), suggesting that anaerobic rather than aerobic metabolism may be crucial to match performance in team sports. This conclusion stems from a previous report that the intron 7 C allele of the *PPARA* gene rs4253778 G/C polymorphism was most frequent in power-oriented athletes (Ahmetov et al., 2006; Ginevičiene et al., 2010), presumably due to hypertrophic effects on skeletal muscle and an increased proportion of fast-twitch muscle fibers (Ahmetov et al., 2013).

The second study (Eynon et al., 2014) analyzed the distribution of the actinin alpha 3 (gene/pseudogene) (*ACTN3*) R577X (rs1815739) polymorphism in 205 team sports athletes from Russia, Poland, and Spain. The sample comprised elite or national

competitive athletes recruited from the following sporting disciplines: football ($n=53$), handball ($n=57$), ice hockey ($n=84$), field hockey ($n=9$), and water polo ($n=2$). The authors found that team sport athletes were less likely to have the 577RR genotype compared to the 577XX genotype than sprint/power athletes [odds ratio (OR): 0.58, 95% confidence interval (CI): 0.34–0.99, $P=0.045$]. However, the *ACTN3* R577X polymorphism was not associated with team sports athletic status when compared to endurance athletes and nonathletic controls.

The study of Eynon et al. (2014) has been subsequently replicated with the same results by Massidda et al. (2015b) on 74 Italian team-sport athletes. The sample was composed of elite or national competitive athletes recruited from field hockey ($n=10$) and football ($n=64$). The results confirmed the previous finding that the *ACTN3* R577X polymorphism was not associated with team sport athletic status when compared to endurance athletes and nonathletic controls.

In addition to those three studies, some recent genetic research focused selectively on single team sports. Among them, the most studied team sports are football and rugby, for which there are specific sections within this chapter. Only one study has been performed on a sample composed exclusively by volleyball players, which examined possible association of the R577X polymorphism in the *ACTN3* gene with "explosive" leg muscle power performance (vertical squat and counter-movement jump tests) (Ruiz et al., 2011). The authors analyzed a group of 66 elite volleyball players (31 men and 35 women) and 334 controls (243 men and 91 women) and did not observe any association of the *ACTN3* R577X polymorphism with study phenotypes (all $P>0.05$). Genotype frequencies were not different between volleyball and control groups ($P=0.095$)—there was no association between the *ACTN3* R577X polymorphism and the likelihood of being an elite volleyball player using either the dominant (RR vs RX + XX) or the recessive model (RR + RX vs XX). In summary, these findings suggest that the *ACTN3* R577X polymorphism does not influence explosive leg muscle power in elite volleyball players.

A similar result was reported some years later in a group of 100 basketball players (60 men and 40 women) and 283 nonathletic controls (Garatachea et al., 2014). *ACTN3* genotype distributions did not differ between groups [cases: 37.0% (RR), 42.0% (RX) and 21.0% (XX); controls: 31.8% (RR), 49.8% (RX), and 18.4% (XX); $P=0.353$]. The authors did not observe any association between the *ACTN3* R577X polymorphism and study phenotypes (vertical squat and countermovement jump) in either group, including when they performed analyses separately in men and women. They found no association between the *ACTN3* R577X polymorphism and the likelihood of being an elite basketball player using the dominant or recessive model, and the results remained unaltered when the analyses were adjusted for sex, body mass, height, and age or when performed for men and women separately.

Although *ACTN3* R577X is associated with explosive muscle performance in some other studies and this phenotype is important in the sport of basketball and

volleyball (i.e., during jumps), these studies found no association with leg explosive power in elite basketball and volleyball players or with the likelihood of being this type of athlete.

More recently, the first study on the identification of genetic markers for skill and athleticism in Australian football has been performed by Jacob et al. (Jacob et al., 2018). The authors recruited 30 young, sub-elite players (aged 16–18 years) who completed tests of endurance, power, and technical skill. The 30 research participants is a very low number and the title of the article includes the words "pilot study," so the results should be treated with much caution. Nevertheless, specific polymorphisms in nine genes were assessed for possible associations with athletic and skill traits. The main result reported was that an insertion/deletion (I/D) polymorphism (rs4343) in the angiotensin-converting enzyme (*ACE*) gene was associated with 'all-round' athletic performance and skill. In detail, the D allele was associated with power ($P \leq 0.008$), endurance ($P = 0.001$), and skill assessments ($P \leq 0.003$). In addition, polymorphisms in the brain-derived neurotrophic factor (*BDNF*), dopamine receptor D2 (*DRD2*), and catechol-*O*-methyltransferase (*COMT*) genes were also associated with kicking skill outcomes ($P \leq 0.044$).

5.4 Genetics of football (soccer)

Several genetic studies have been conducted in the field of football identifying different genetic markers associated with players' physical characteristics, such as cardiac morphology and function (Fatini et al., 2000; Rizzo et al., 2003; Saber-Ayad et al., 2014), body composition (Micheli et al., 2011; Massidda et al., 2016), and bone phenotypes (Diogenes et al., 2010; Varley et al., 2018). Some studies have been focused on the potential genetic variants influencing the likelihood of becoming a football player (Egorova et al., 2014) and key factors of football performance (Pimenta et al., 2013). Recently, a few studies have also assessed the association of genetic characteristics with the predisposition of football players to develop musculoskeletal injuries (Massidda et al., 2015a, c, 2019; Artells et al., 2016). Starting with a brief description of the phenotype of interest, the present section provides an overview of the genetic markers associated with the likelihood of becoming a football player and the key factors of football performance.

5.4.1 Phenotype description of football performance

Like other field-based team games, the physiological demands of football are complex as they are related to a mix of both metabolic (e.g., aerobic power/capacity) and neuromuscular (e.g., muscle strength) factors (Bangsbo, 1994a, b). A football match is played by two teams comprising no more than 22 players on the pitch (11 for each team), including two goalkeepers (one for each team) and 20 outfield players (10 for each team). A match is played in two 45 min halves separated by 15 min of passive recovery time. The aim of the

game is to score a goal, which is achieved by kicking or heading the ball into the opposing team's goal. Each player has a set position and a specific role to perform in the course of a 90-min game of football. The main positional roles are represented by a goalkeeper, defenders, midfielders, and forwards. However, within the same positional roles there are more specific roles depending on the system of play and the responsibility of the players during the game (e.g., Forwards: center forward, second strikers, winger; Midfielders: defensive midfield, center midfield, wide midfield, attacking midfield; Defenders: center back; sweeper; full-back; wing-back).

The activity profile and demands on a player are determined by the positional role in the team. For example, central defenders commonly cover less total distance and engage in less high-intensity running than players in the other positions, which is probably closely linked to their tactical roles and their lower physical capacity in certain respects (Bangsbo, 1994a, b; Krustrup et al., 2003; Mohr et al., 2003). The highest sprinting activity is a characteristic of forwards, who usually run almost twice as many sprints per game as central midfielders and central defenders (Di Salvo et al., 2007).

Among the several skills required for an optimal football performance, the RSA represent a highly significant key factor in modern football (Bishop et al., 2004) and the amount of high-speed running distinguishes top-class players from those at a lower level (Bangsbo, 2014). Forwards generally have significantly better RSA than defenders and midfielders (Aziz et al., 2008).

Playing formation does not influence the overall activity profiles of players, except for forwards, but does have an impact on very high-intensity running activity, with and without ball possession, and some technical elements of performance (Bradley et al., 2011).

Although players perform low intensity activities for more than 70% of the game, they cover a distance of about 10–12 km (Bangsbo et al., 1991; Ohashi et al., 1988; Van Gool et al., 1988; Withers, 1982) at an average intensity close to the anaerobic threshold, being 80%–90% of maximal heart rate (HR_{max}) or 70%–80% of maximal oxygen uptake (VO_{2max}) (Helgerud et al., 2001; Reilly, 1994b). The rate of muscle lactate production is high during match play, with an average blood lactate concentration of 2–10 mM (Bangsbo et al., 1991; Ekblom, 1986; Krustrup et al., 2006; Tessitore et al., 2005). Muscle glycogen seems to be the most important substrate for energy production since different studies observed that muscle glycogen stores in a significant number of muscle fibers were depleted or partly depleted at the end of the game (Saltin, 1973; Krustrup et al., 2006). Moreover, the oxidation of fat appears to increase progressively during a game and more so during the second half, partially compensating for the progressive lowering of muscle glycogen (Bangsbo, 1994a, b; Krustrup et al., 2006).

Finally, several factors influence the activity of a player during a match, such as the player's physical capacity, technical qualities, playing position, tactical role, and style of playing, as well as ball possession of the team, quality of the opponent, importance

of the game, seasonal period, playing surface, and environmental factors (Bangsbo, 2014). Referring to genomic studies, a careful selection of research participants is required, in terms of technical level and positional roles, due to those factors influencing the physical demands of a football player.

5.4.2 Genetic markers associated with athletic status and key phenotypes in football

5.4.2.1 Case-control studies

The studies focused on the potential genetic variants influencing the athletic status of football player suggest that the likelihood of becoming a good player depends on the carriage of a high number of "favorable" gene variants (Egorova et al., 2014; Gineviˇciene et al., 2014). Similar to other sports and athletic performances, the most studied genetic markers in the field of football are *ACTN3* R577X and *ACE* I/D polymorphisms.

The first evidence for an association between *ACTN3* and football performance was published in 2008 by Santiago et al. (2008). The authors studied a small group of 60 top-level Spanish professional soccer players and found a significantly higher frequency of *ACTN3* 577RR genotype in football players than in controls and endurance athletes. The study has been subsequently replicated with the same results in a larger cohort of 246 Russian football players (all playing positions and separately in forwards) (Egorova et al., 2014), in a very small sample of 25 professional Turkish football players (Ulucan et al., 2015) and, more recently, in a small group of 45 male football players from a 2nd division team of the Italian National League (Galeandro et al., 2017). Since the *ACTN3* R allele is usually overrepresented in sprint and power-oriented athletes, all these studies suggest that football players tend to have a sprint/power-oriented genotype. Conversely, Massidda et al. (2014) analyzed the distribution of *ACTN3* polymorphism in a cohort of 90 Italian top-level football players and did not find any significant difference in genotypic distribution between players and controls.

Finally, a recent study has investigated a possible role of the *ACTN3* R577X polymorphism on a football player's career progression (Coelho et al., 2018). The study included 353 players from first division Brazilian football clubs (under-14;U-14, U-15, U-17, U-20 and professional). The XX genotype was less frequent among professional players than in the U-20 ($P<0.05$) or U-15 categories ($P<0.05$). The RX genotype was also more frequent in the professional category than the U-14 category ($P<0.05$). Moreover, a trend toward a higher frequency of the RX genotype and a lower frequency of the XX genotype was observed in the professional category compared to U-20. These results suggest that the genotype in the *ACTN3* polymorphism affects the probability of a football player progressing throughout his career and becoming professional, meaning that playing football selects against the *ACTN3* XX genotype.

Although not all studies found an association between *ACTN3* polymorphism and football player status, there is overall a tentative consensus that the R allele and RR

genotype can impact the phenotype of interest, influencing the likelihood of becoming a football player, independent of players' ethnicity.

Regarding the *ACE* I/D polymorphism, the first study was carried out in 2009 by Juffer et al. (2009) on a group of 54 male professional football players. The authors found a higher frequency of ID genotype and a lower frequency of II genotype in football players than in endurance runners (Juffer et al., 2009). Several years later, the study was replicated with the same result (an excess of the ID genotype) in a group of 199 Lithuanian football players (Ginevičiene et al., 2014). In addition, Egorova et al. (2014) examined a larger cohort of 246 Russian football players reporting a higher frequency of the D allele and DD genotype in players compared to controls (all playing positions and separately in goalkeepers). The study was also replicated in a very small study on 25 Senior Turkish football players (Ulucan et al., 2015) and in 43 Italian professional football players (Galeandro et al., 2017). Since the *ACE* D allele is overrepresented in some (though not all) studies of sprint/power-oriented athletes, these findings might suggest that football players tend to have a sprint/power-oriented genotype. On the other hand, three more studies conducted, respectively, on Italian (Massidda et al., 2012), Polish (Cięszczyk et al., 2016a), and Brazilian (Coelho et al., 2016) football players did not find any significant difference in *ACE* I/D genotype frequency distribution between football players and controls. More research is needed to clarify the role of the *ACE* I/D polymorphism in football with respect to different playing positions.

More genetic variants have been associated with football player status in few more studies even if they have not yet been replicated in other cohorts with different geographic ancestry. In detail, the frequency of the C allele of the *PPARA* rs4253778 polymorphism was higher in a cohort of 246 Russian football players (all playing positions and separately in forwards) (Egorova et al., 2014) than controls while the CC genotype was more prevalent in 44 Lithuanian forward football players (Ginevičiene et al., 2014) compared to controls. Considering that the *PPARA* C allele has been overrepresented in power/strength-oriented athletes and associated with an increased proportion of fast-twitch muscle fibers, these findings support the notion that football players tend to have a power/strength-oriented genotype. In the same study of Russian football players, Egorova et al. (2014) found an elevated frequency of the *UCP2* 55Val allele (all playing positions and separately in defenders and midfielders). This allele is potentially associated with increased exercise efficiency and aerobic performance (Buemann et al., 2001; Ahmetov et al., 2008).

Moreover, the likelihood of having the C allele of the *NOS3* −*786* T/C polymorphism (rs2070744) was higher in 60 male professional Spanish elite soccer players compared with (i) controls [odds ratio (OR), 2.165, 95% confidence interval (CI): 1.362–3.441], (ii) endurance athletes (OR: 1.879, 95% CI: 1.184–2.984), and (iii) power athletes (OR: 4.032, 95% CI: 2.307–7.047) (Eynon et al., 2012b).

In a recent study on a large number of top-level football players ($n=694$) from five different European countries (Massidda et al., 2018) the A allele and the AA genotype of the *MCT1* rs1049434 polymorphism, previously correlated with a higher lactate clearance (Cupeiro et al., 2010, 2016; Fedotovskaya et al., 2014), was associated with forward football player status. Interestingly, an increased frequency of the *MCT1* A allele (92.9 vs 62.5%, $P=0.023$) was also observed in Russian volleyball players compared to controls (Fedotovskaya et al., 2014). This study emphasized the importance of considering football players' positional roles in genotype-phenotype association studies and, consequently, the need to use a large cohort of players to be more confident in the statistical results.

To quantify the combined influence of four candidate polymorphisms (*ACE* I/D, *ACTN3* R577X, *PPARA* intron 7 G/C, and *UCP2* A55V) on football player's status, Egorova et al. (2014) used an algorithm (Hughes et al., 2011; Williams and Folland, 2008) to incorporate all favorable genotype scores for any given individual in a simple additive model. The total genotype score (TGS) calculated from the accumulated combination of the four polymorphisms (with a maximum value of 100 for the theoretically optimal polygenic score) was significantly higher in a cohort of 246 Russian football players [mean (standard deviation): 52.0 (17.6) vs 41.3 (15.5); $P<0.0001$] than in controls. These data suggest that the likelihood of becoming a football player depends on the carriage of a high number of "favorable" gene variants.

5.4.2.2 Genotype-phenotype studies

The current literature regarding genetic characteristics associated with measured phenotypes relevant to football performance is also discordant. The main phenotypic characteristics of modern football performance considered in genetic studies are vertical jumps, sprint performance, RSA, and VO_{2max}.

Regarding the vertical jumps, the first study was conducted to explore the role of the *ACE* I/D polymorphism on performance variation within players. A total of 125 medium-high level male Italian football players (Micheli et al., 2011) were recruited for the study and their Squat Jump (SJ) and Counter Movement Jump (CMJ) recorded. Carriers of the ID genotype achieved higher vertical jumps.

Vertical jump performance has also been associated with the *ACTN3* polymorphism. In 42 Italian football players (Massidda et al., 2012), a multivariate model combining genotypic data (*ACE* and *ACTN3* polymorphisms) and competition level of the players significantly predicted vertical jump height (measured by SJ and CMJ).

Pimenta et al. (2013), studying a cohort of two hundred professional players of Brazilian soccer first division teams, found that individuals of *ACTN3* RR and RX genotype presented the higher data in jump tests compared with XX genotypes. That study has been replicated recently by Dionísio et al. (2017) in a group of 220 young male athletes from professional minor league soccer team from São Paulo Futebol Clube, Brazil.

Sprint performance, measured using 10 m, 20 m, and 30 m sprint tests, has been associated with the *ACTN3* (Pimenta et al., 2013), *ACE,* and the *AMPD1* genes (Dionísio et al., 2017). In detail, football players with *ACTN3* RR and RX genotypes and *ACE* DD genotype were faster than those with *ACTN3* XX genotype and players with *ACE* II genotype (Pimenta et al., 2013; Dionísio et al., 2017), while the players with *AMPD1* CC genotype were only favored in the 10-m sprint test (Dionísio et al., 2017).

Conversely, football players with *ACTN3* XX genotype and *ACE* ID/II genotypes seemed to exhibit a higher VO_{2max} and to have a better endurance performance than players with *ACTN3* RR genotype and *ACE* DD genotype, respectively (Pimenta et al., 2013; Dionísio et al., 2017).

The main conclusion of these studies was that football players with *ACTN3*/RR, *ACE*/DD, and *AMPD1*/CC genotypes were the fastest in short distances and were able to jump higher, while *ACTN3*/XX and *ACE*/II individuals showed the highest aerobic capacity.

Not all studies on *ACTN3* report consistent findings. Coelho et al. (Coelho et al., 2016), for example, investigated the association between the *ACTN3* polymorphism and physical performance (vertical jumps, speed, and VO_{2max}) of 138 adult professional U-20 and U-17 years Brazilian first-division soccer players. No significant differences were observed in genotype or allele frequencies between different performance ratings, demonstrating that *ACTN3* polymorphism was not associated with any of the physical performance parameters.

These conflicting results should be explored deeper in further study. Here we could hypothesize that the differences in competition level of the players, in the distributions of the players' positions within samples, in geographic ancestry and in the training methodologies could have produced the conflicting results observed in the literature.

5.4.3 Conclusion and future research directions

In the past few years, several genetic studies have been conducted in the field of football with the aim to discover genetic variations associated with football performance and the likelihood to become an elite football player. While there have been some advances in knowledge of genes associated with phenotypes relevant to football, more and better-designed studies are needed to overcome the studies' limitations carried out so far.

One of the major limitations in the field of football genomics is the relatively low sample size of players participating in the studies. The major collaborative effort is required for the field to progress and enhance our understanding of the genes that influence the likelihood of becoming a football player and the response to football training.

Regarding the contrasting results obtained in the field of the genetic influence on football player status, it is important to highlight the mixed nature of football performance in which players are required to possess a balance between aerobic and anaerobic

fitness that can make it more difficult to discover significant genetic associations with their athletic status (Eynon et al., 2014; Massidda et al., 2015b). Moreover, the validity of this form of data is affected when dealing with team sports, since each football player's physical demands also differ according to the position on the team (Sporis et al., 2009; Mendez-Villanueva et al., 2011). It has already been argued that heterogeneity occurs in the anthropometric characteristics and physiological variables of football players on elite teams and that various factors can predispose players to success (Reilly et al., 2000).

Finally, a lack of consistency appears when comparing the results of the studies focused on the genetic markers associated with relevant phenotypes in football. In fact, the different training protocols adopted by the teams and the heterogeneity of the competition level of the samples recruited in different studies make it difficult to compare results between studies.

In conclusion, genetic information might, in future, be used by coaches to enable footballers to gain the greatest benefit from their training program. However, even after knowing that genetic variation in football has advanced further, it will be important to keep in mind that football performance is a very complex multifactorial trait influenced by many genetic variations, environmental factors, and the interaction among them and that, in order for genetic testing to become a useful component of football training practice, intensive international collaboration will be required.

5.5 Genetics of Rugby

RU is normally played by two teams of 15 athletes (eight forwards and seven backs) and rugby league (RL) by two teams of 13 athletes (six forwards and seven backs). In both codes of rugby, each athlete has a specific role and the role can differ between codes even if the position has the same name (e.g., hooker). There are many similarities in physical characteristics and movement patterns between RU and RL athletes. However, RL has no lineouts, rucks or mauls and no more than six tackles during one period of ball possession. A substantial proportion of the interindividual variation for many rugby-related performance traits, including muscle strength, peak power output, the maximal rate of oxygen uptake, injury susceptibility, and the likelihood of being an elite athlete is inherited and can be investigated using molecular genetic techniques. In particular, the RugbyGene project has made some early progress in describing genetic characteristics associated with elite rugby athlete status or relevant traits. This section provides a short review of this area and a commentary regarding the directions that research in this field should now take.

5.5.1 Phenotype description of rugby performance

Each rugby athlete has a designated position that requires specific physical and technical characteristics (Gabbett, 2005; Mellalieu et al., 2008; Brazier et al., 2018). RU forwards

are involved in more scrums, lineouts, rucks, and mauls, which demands greater height, mass, power, and strength (Duthie et al., 2006), while the backs' main role in open play requires a combination of speed, acceleration, and agility (Duthie et al., 2003), thus power and strength relative to body mass. This is similar in RL where forwards perform a high number of tackles, whereas backs perform freer running (Gabbett et al., 2008).

RU and RL require elite athletes to frequently perform intense activity, such as tackling, wrestling, sprinting, and running, combined with short periods of less intense activities, such as walking and jogging (Brewer and Davis, 1995; Brooks and Kemp, 2008; Gabbett et al., 2008; Mellalieu et al., 2008). In RL, outside backs (winger, fullback, and center) cover most distance (~6800 m), followed by adjustables (halfback, standoff, and hooker), wide-running forwards, and hit up forwards (prop, second row, and loose forward) (~3500 m) (Gabbett, 2012). This is similar in RU, with front row forwards covering least distance (~4900 m), increasing through the second row forwards and back row forwards to outside backs and inside backs (~6100 m), with back row and front row forwards performing more high-intensity exercise than backs (Austin et al., 2011; Jones et al., 2015).

Rugby collisions occur during tackles, offensive hit-ups and from clearing rucks, mauling, mid-air contact, and falls (Gabbett et al., 2007; Fuller et al., 2013). Impact forces for RL hit-up forwards can be >10 *g* every 2 min of match play, but of much lower magnitude and frequency for outside backs (Cummins and Orr, 2015). Similar collision rates exist in RU, with more for forwards (0.7–0.9 collisions per min) than backs (0.3–0.4 collisions per min) (Reardon et al., 2017). In RU, the number of tackles made per match ranges from ~29 (back row) to ~16 (outside backs) (Deutsch et al., 2007), where injury risk is higher for ball carriers than tacklers and when being tackled by two or more opponents (Quarrie and Hopkins, 2008). In RL, forwards experience ~55 collisions (39 tackles, 16 hit-ups) and backs ~29 (16 tackles, 13 hit-ups) per match (Gissane et al., 2001).

There are relationships between anthropometric and physiological characteristics and in-game tasks in both RU and RL (Gabbett et al., 2007; Gabbett et al., 2013; Smart et al., 2014). For example, 10–30 m sprint times were correlated (fairly weakly) with line breaks ($r \sim 0.26$), meters advanced ($r \sim 0.22$), tackle breaks ($r \sim 0.16$), and tries scored ($r \sim 0.15$) per game in elite RU. In RL, the ability to offload from a tackle was associated with higher body mass ($\eta = 0.474$), whereas the ability to beat an opponent using speed and agility was associated with lower skinfold thicknesses ($\eta = -0.454$), higher vertical jump height ($\eta = 0.442$), better agility, and faster 20–40 m sprint times ($\eta = -0.467$ and $\eta = -0.483$, respectively).

Given the relationships described above, it is not surprising that compared to lower competitive standards, elite athletes are the heaviest (RU forwards ~111 kg, backs ~93 kg; RL forwards ~103 kg, and backs ~90 kg), have lowest % body fat (RU forwards ~15%, backs ~12%; RL forwards ~14%, and backs ~11%), have most fat-free

mass, are strongest (back squat: RU forwards ~176 kg, backs ~157 kg; RL forwards ~188 kg, backs ~ 168 kg; bench press: RU forwards ~131 kg, backs ~118 kg; RL forwards ~122 kg, and backs ~113 kg), and fastest (10 m: RU forwards ~1.87 s, backs ~1.77 s; 10 m RL forwards ~1.9 s, and backs ~1.83 s) (Gabbett, 2002; Duthie et al., 2003; Gabbett, 2006; Lundy et al., 2006; Till et al., 2011; Sedeaud et al., 2012; Fuller et al., 2013; Sedeaud et al., 2013; de Lacey et al., 2014; Morehen et al., 2015; Till et al., 2017; Brazier et al., 2018). Indeed, some of those data probably underestimate the physical qualities of contemporary athletes (Brazier et al., 2018). Thus, RU and RL elite athletes have high maximal aerobic and anaerobic power, speed, agility, and muscular strength and power, as well as the underlying anthropometric features to provide those functional abilities. All those physiological and anthropometric characteristics will result from a combination of environmental (training, diet, etc.) and genetic factors.

5.5.2 Genetic markers associated with athletic status and key phenotypes in rugby

Almost a century ago, Jack (1922) described the playing positions of 23 sets of elite rugby-playing brothers and concluded that "*the ability required for playing in certain positions in rugby football is inherited*." It took nearly 90 years for the first molecular genetic studies of rugby to appear. A very small study using just 17 participants reported that *ACE* II genotype was associated with a higher ventilatory threshold in non-elite Asian RU athletes (Goh et al., 2009). In Wales, Bell et al. determined *ACE* I/D and *ACTN3*R577X genotypes in 68, and 102 young non-elite RU athletes, respectively (Bell et al., 2010, 2012). No associations were observed between polymorphism and athlete status, playing a position or physiological and anthropometric parameters, probably due to the rather small sample size and use of sub-elite athletes.

Subsequently, the RugbyGene study was established, which is an ongoing, large, multi-institutional effort to make progress in this field (Heffernan et al., 2015). That study uses a current definition of 'elite' as athletes who competed regularly (> 5 matches) since 1995 in the highest competitive league of a 'Tier 1' nation for RU and the highest professional league in the UK or Australia for RL. RU and RL have changed dramatically throughout their histories, with changes in RU particularly rapid around the time the sport turned professional in 1995—hence that year was chosen as a playing era inclusion criterion for both RU and RL. The recent publications from RugbyGene are described in the following text.

Heffernan et al. (2016) evaluated the most frequently studied variants in sport and exercise genetics (*ACE* I/D and *ACTN3* R577X) in 507 Caucasian elite male rugby athletes (431 RU and 83 RL; a small number of athletes competed in both codes) and 710 Caucasian nonathletes. There was no difference in *ACE* I/D genotype between groups but *ACTN3* XX genotype tended to be underrepresented in RU backs (15.7%) compared with forwards (24.8%, $P=0.06$). Interestingly, the 69 back three

players (wings and full backs) in RU included only six *ACTN3* XX individuals (8.7%), with the R allele more common in the back three (68.8%) than controls (58.0%; odds ratio 1.60) and forwards (47.5%; odds ratio 2.00). These results differed from the previous comparable study (Bell et al., 2012), probably because a much larger and more elite athlete sample was used by Heffernan et al. Association of *ACTN3* R577X with playing position in elite RU suggests inherited fatigue resistance is more prevalent in forwards, while inherited sprint ability is more prevalent in backs, especially wings and full backs. For the first time, these results also demonstrated the advantage of studying a large cohort in a single sport, especially when intra-sport positional differences exist, instead of combining several disparate sports.

In 1089 participants comprising 530 Caucasian elite male rugby athletes (450 RU and 88 RL; a small number of athletes competed in both codes) and 559 Caucasian nonathletes, Heffernan et al. (2017a) evaluated the *FTO* rs9939609 variant that was previously associated with obesity-related phenotypes in nonathlete populations. In a subgroup of nonresistance trained individuals (NT; $n=120$), skeletal muscle phenotypes were assessed using dual-energy X-ray absorptiometry, ultrasound, and isokinetic dynamometry. In a subgroup of RU athletes ($n=77$), muscle power was assessed using a countermovement jump. In NT, TT genotype and T allele carriers had greater total body (4.8% and 4.1%) and total appendicular lean mass (LM; 3.0% and 2.1%) than AA genotype, with greater arm LM (0.8%) in T allele carriers and leg LM (2.1%) for TT genotype compared to AA. The T allele was also more common (94%) in selected elite RU athletes (back three and center players) who rely more on LM than total body mass for success, compared to other rugby athletes (82%; odds ratio = 3.34) and controls (84%; odds ratio = 2.88). The athletes specializing in those outside back playing positions also had greater peak power relative to body mass than other rugby athletes (14%; $P=2\times10^{-6}$). The study suggests that the *FTO* T allele is associated with increased LM and career success for RU athletes who compete in outside back positions.

Heffernan et al. (Heffernan et al., 2017b) studied two SNPs in the *COL5A1* gene (rs12722 C/T and rs3196378 C/A) previously associated with tendon and ligament pathologies in 1105 participants comprising 460 elite RU, 88 elite RL athletes, and 565 nonathletes. For rs12722, the injury-protective CC genotype and C allele were overrepresented in all rugby athletes (21% and 47%, respectively) and RU athletes (22% and 48%) compared to nonathletes (16% and 41%, $P\leq0.01$). Similarly, for rs3196378, the CC genotype and C allele were more common in all rugby athletes (23% and 48%) and RU athletes (24% and 49%) than nonathletes (16% and 41%, $P\leq0.02$). The CC genotype, in particular, was overrepresented in the back three and centers (24%) compared with nonathletes (16%; odds ratio = 2.25). More powerfully, when considering both SNPs simultaneously, the CC—CC SNP-SNP and C—C inferred allele combinations were higher in all athlete groups (≥18% and ≥43%) than nonathletes (13% and 40%; $P=0.01$). No genotype differences were identified for either

SNP when RU playing positions were compared. It appears that the C alleles, CC genotypes, and resulting combinations of both rs12722 and rs3196378 are advantageous for rugby athletes and possessing these variants may provide inherited resistance against soft tissue injury, despite exposure to the high-risk environment of elite rugby. Combining genetic data from multiple gene variants associated with injury susceptibility, such as those presented here, with nongenetic indicators of injury risk and recovery during rehabilitation could be used to better manage the prevention of and recovery from musculoskeletal injury in the future.

Notable and promising research into the genetics of brain injury including concussion has also used a rugby cohort (Abrahams et al., 2018; Mc Fie et al., 2018a, b). However, that research is not discussed in detail here in this chapter because it is not focused on a rugby performance phenotype or elite status and will be addressed in a later chapter of this book.

5.5.3 Conclusion and future research directions

Some promising progress has been made recently in research into the genetics of rugby performance, elite rugby athlete status, and relevant phenotypes, as described above. However, there is very much more yet to be discovered than is understood at present. Increasing elite athlete sample sizes will be key for the scale of genetic analyses to be increased toward using hypothesis-free approaches in addition to candidate gene studies. Therefore, thousands of elite rugby athletes will be required for a project like RugbyGene to fulfill its full potential and that will only be achieved through continued and accelerated international research collaboration and the application of emerging molecular technologies appropriate to the scale of phenotype data available (Pitsiladis et al., 2013, 2016; Heffernan et al., 2015; Wang et al., 2016).

Additional steps should be taken to investigate genotype associations with phenotypes of most relevance to practitioners in rugby—that is, not only overall success in rugby (elite athlete status and playing position) or even the physiological, anthropometric, and other performance variables measured in the laboratory or in the field. Probably the most direct evidence of the relevance of genetic data to rugby performance and injury susceptibility would emerge from a simultaneous analysis of genetic and in-game phenotypic (performance and injury) data. Excellent rugby injury surveys are already well-established (Fuller et al., 2017; England Professional Rugby Injury Surveillance Project Steering Group, 2018; Fitzpatrick et al., 2018) while in-game match performance statistics (number of tackles completed, meters gained carrying the ball, etc.) also exist and associations of genetic data with those 'extended phenotypes' would resonate strongly with rugby practitioners. The challenge will then be to apply genetic technologies, quite obviously alongside (not instead of) existing nongenetic data, to personalize the management of players in rugby and facilitate the prescription of training, nutrition, playing load,

and the management of injury risk in a more personalized way than is currently possible to improve both on-field performance and player welfare.

Thus far, it is obvious that almost all published research on elite rugby athletes has involved male participants only. However, women's rugby (RU and RL) has recently gained a higher profile in international competition, in full and shorter versions of the game [e.g., Rugby Sevens (RU) at the Olympic Games]. Professional club competition in women's rugby is also beginning to emerge. The heightened interest and levels of participation and competition in women's rugby mean that research into the genetics of elite rugby performance in women is also becoming worthwhile and viable.

Geographic ancestry is an important consideration for case-control and genotype-phenotype studies and analysis of molecular genetic markers would ideally be conducted with athletes from a well-defined geographic ancestry cluster. Indeed, that is the approach taken so far by researchers studying the genetics of rugby. It will be very challenging to recruit large numbers of players from all major geographic ancestry clusters commonly found in RU, although this would be a very powerful approach scientifically and it should form part of this field's future research landscape.

5.6 Conclusion

To date, genetic research in the field of team sports has produced some advances in knowledge of genes associated with team sports performance, team sports athlete status, and some relevant phenotypes. However, despite the great potential of the genetic studies in team sports, we are still in the opening stages of the research and there is very much more yet to be discovered. Candidate gene studies focusing predominantly on genotyping a limited number of genetic variants in small and heterogeneous cohorts has not generated results of broad practical utility.

Different opinions in the classification of team sports and in the competition level of the athletes have generated confusion and hindered progress in this field. Moreover, the lack of replication studies limits the ability to determine if experimental results are robust enough and reveal a real phenomenon, or if they are merely highly specific conditions that are difficult to recreate. In view of the foregoing, it is essential to undertake a few important steps for future research. First of all, in consideration of the fact that within the category of team sports, the demands between sports is widely variable, it is essential to examine a large cohort of athletes in a single sport.

With regard to the intra-sport positional differences in physiological and technical demands, it is important to analyze genetic associations between and among positional roles. The real advantage of studying intra-sport positional differences is exemplified by the recent studies on rugby and football that revealed a significant difference in genotype distribution among positions on the field (Heffernan et al., 2016; Massidda et al., 2018). Moreover, particular attention should be also placed to the competition level

of the athletes, referring to the category accepted and utilized by International Federations. Finally, in addition to overall success in team sports (i.e., elite athlete status and playing position), measurable phenotypes of most relevance to practitioners in each team sport should be incorporated into study designs.

Creating substantial databases with both genotypic and phenotypic variables, such as physiological, anthropometric, and other performance variables measured in the laboratory and in the field is necessary to get a clear picture of the role of genetic characteristics on team sports performance. Therefore, large collaborative projects with sound experimental designs (i.e., clearly defined phenotypes, consideration, and control of sources of variability, and necessary replications) are required to produce meaningful results in a practical sense.

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CHAPTER SIX

Variation of Mitochondrial DNA and elite athletic performance

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6.1 Introduction

In eukaryotes, mitochondria provide the majority of adenosine triphosphate (ATP) required for sustaining life and play an important role in the physical performance of humans. The ATP required for muscle contraction during exercise and sports are provided by creatine/creatine-phosphate and glycolytic systems in the cytoplasm and oxidative phosphorylation (OXPHOS) system in the mitochondria. Among them, mitochondrial OXPHOS is the most efficient energy-producing system, providing 36 molecules of ATP per glucose molecule in contrast to the 2 ATP molecules produced by glycolysis; OXPHOS is also able to use lipids and amino acids. Especially for endurance-type exercises, ATP synthesized by mitochondrial OXPHOS is the main source of energy. Endurance training increases mitochondrial content in the skeletal muscle (Holloszy, 1967). An increase in the number of muscle mitochondria is associated with the improvement in ATP production, conservation of glycogen usage due to the preferential use of lipids as the energy source, and the suppression of lactate accumulation; all of these factors culminate in enhancing endurance capacity (Holloszy and Coyle, 1984).

Although the majority of mitochondrial proteins (>1000) are encoded by the nuclear genes, 13 subunits of the OXPHOS system are encoded by the mitochondrial DNA (mtDNA). Hence, it is possible that variations of mtDNA influence the capacity and/or efficiency of ATP production by the OXPHOS system, and therefore, exercise performance. To date, many studies have suggested that variations of mitochondrial genome influence the mitochondrial function and elite athletic performance. In this chapter, we summarize the findings regarding the association of the variations of the mitochondrial genome with elite athletic performance.

6.2 Characteristics of mitochondrial DNA (mtDNA)

Mitochondria are thought to have originated in bacteria that were subsequently engulfed by ancestral eukaryotic cells; therefore, mitochondria have their own genome

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in the form of a circular DNA molecule. The double-stranded circular human mtDNA is 16,569 base pairs (bp) in length, and contains 37 genes for 13 proteins of mitochondrial OXPHOS, two ribosomal RNAs (rRNAs), and 22 transfer RNAs (tRNAs) that are necessary for protein synthesis within the mitochondria (Anderson et al., 1981) (Fig. 6.1). The mitochondrial OXPHOS system is composed of five enzyme complexes (Complexes I–V), of which four (Complexes I, III, IV, and V) contain subunits encoded by the mtDNA (Fig. 6.2). Complex I (NADH dehydrogenase) contains NADH dehydrogenase subunits (ND)1, ND2, ND3, ND4, ND4L, ND5, and ND6; complex III (bc1 complex) contains cytochrome b (Cytb); complex IV (Cytochrome c oxidase) contains

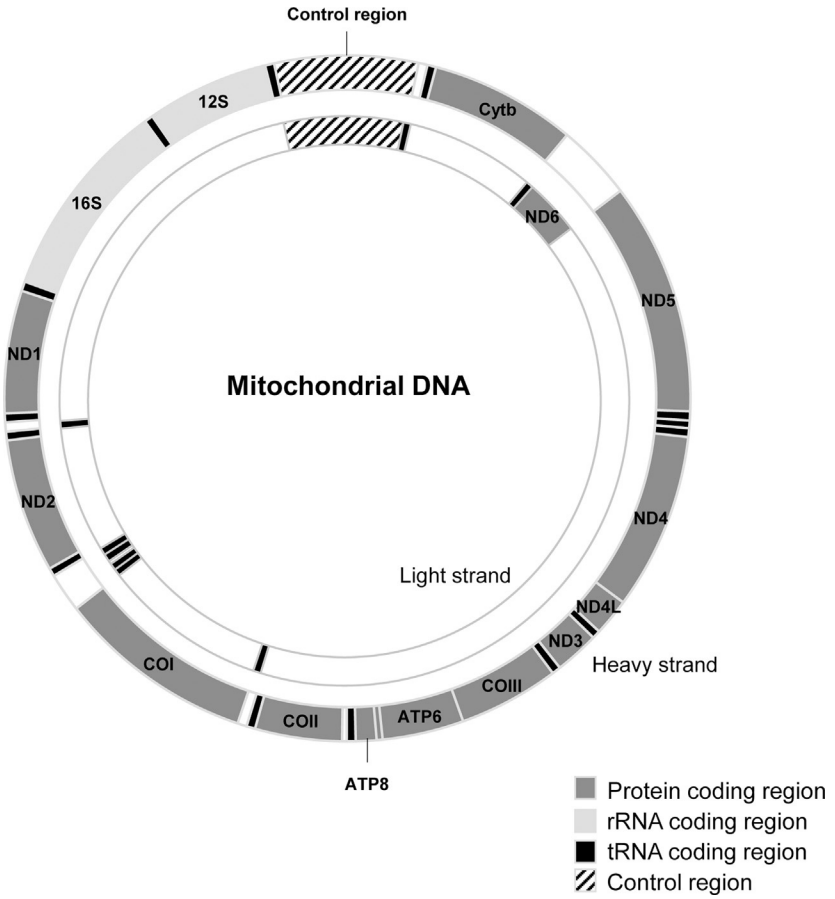

Fig. 6.1 The human mitochondrial DNA (mtDNA). Human mtDNA encode 13 polypeptides: 7 subunits (ND1, ND2, ND3, ND4, ND4L, ND5, and ND6) of Complex I, 1 subunit (Cytb) of Complex III, 3 subunits (COI, COII, and COIII) of Complex IV, and 2 subunits (ATP6 and ATP8) of Complex V. ND: NADH dehydrogenase subunit; Cytb: Cytochrome b; CO: Cytochrome c oxidase subunit; ATP: ATP synthase subunit; 12S: 12S ribosomal RNA; 16S: 16S ribosomal RNA.

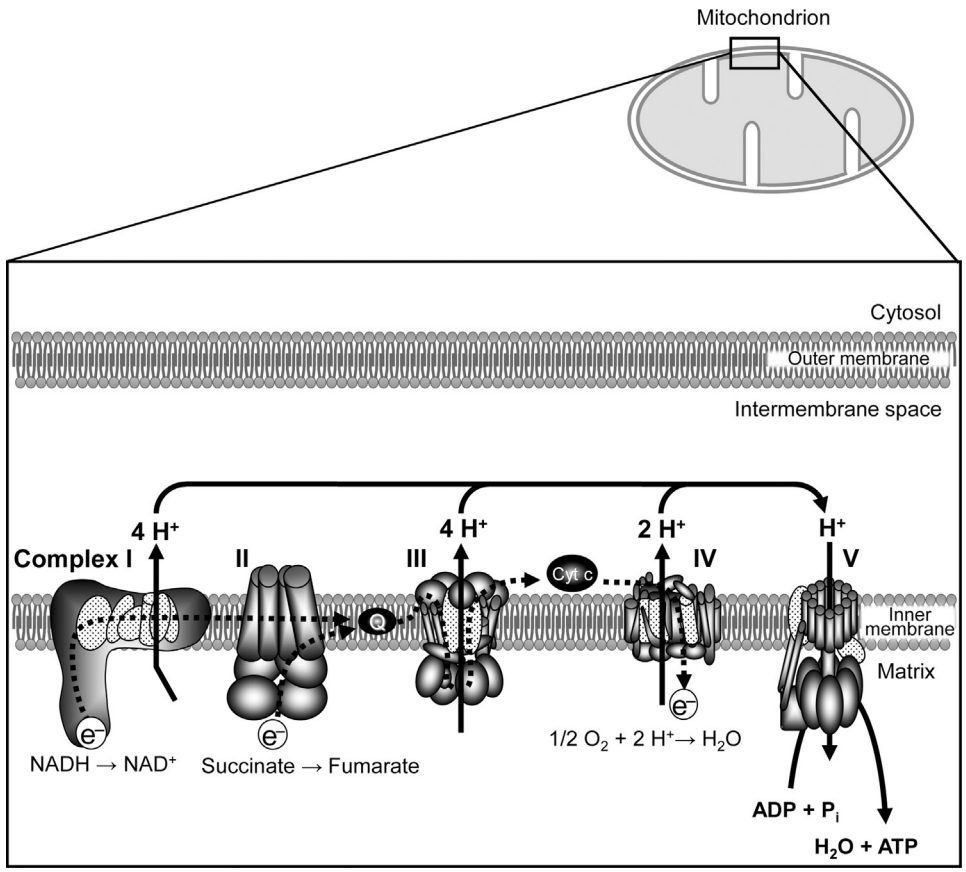

Fig. 6.2 Mitochondrial oxidative phosphorylation system. Thirteen subunits encoded by mtDNA were shown in pin-dot pattern.

cytochrome c oxidase subunit (CO)I, COII, and COIII; and complex V (ATP synthase) contains ATP synthase subunit (ATP)6 and ATP8 encoded by mtDNA. All mitochondrial proteins except these 13 subunits are encoded by nuclear DNA; therefore, the mitochondrial function requires the coordinated expression of both nuclear DNA and mtDNA. Although the 13 mtDNA-encoded subunits constitute only a minority of OXPHOS, they are essential for the function of OXPHOS. It was demonstrated that the OXPHOS system ceases to function in the absence of mtDNA (Larsson et al., 1998), and that sequence variations of mtDNA influence OXPHOS performance (Moreno-Loshuertos et al., 2006).

The two strands of mtDNA are different in their base composition; the strand rich in guanines is called the heavy (H) strand and the other, light (L) strand. The H strand encodes most of the genes (Fig. 6.1). One of the characteristics of mtDNA is its compact organization, where all the genes are contiguous to each other or separated by a few bases

and have no intron. There are only two noncoding regions in mtDNA, namely major and minor noncoding regions (Fernandez-Silva et al., 2003). The major noncoding region is located between the genes for phenylalanine and proline tRNAs, and is called the control region (Fig. 6.1). The control region contains the regulatory elements for mtDNA transcription and replication.

Unlike nuclear DNA, mtDNA is exclusively maternally inherited (Giles et al., 1980); that is, only mothers transmit their mtDNA to their children. There is a mechanism for the active degradation of paternal mitochondria (Sato and Sato, 2011). Interindividual differences in exercise performance are influenced by both environmental and genetic factors. It was demonstrated that almost half of the variations in endurance capacity and muscle strength that are intermediate phenotypes of athletic performance are determined by genetic factors (Miyamoto-Mikami et al., 2018; Zempo et al., 2017). Athlete status is also significantly affected by genetic factors (De Moor et al., 2007). However, the genetic variants contributing to interindividual differences in athletic performance largely remain unclear. Several familial studies have reported that aerobic capacity and its trainability have a stronger maternal inheritance than paternal (Bouchard et al., 1998, 1999). Therefore, maternally inherited mtDNA is likely to contain genetic markers influencing aerobic capacity, and therefore, potentially elite athletic performance.

6.3 Early studies of mtDNA polymorphisms and exercise performance

Approximately 20 years ago, Dionne et al. first reported the association between mtDNA polymorphism and exercise performance in 1991 (Dionne et al., 1991), wherein they examined the association of mtDNA polymorphisms with interindividual variations in $\dot{V}O_{2max}$ and its response to 20-week endurance training in 46 sedentary young adult males. From the analysis of 22 restriction fragment length polymorphisms (RFLPs), they found that three RFLPs (*Bam*HI-morph 3 in the *ND5* gene [m.13470A>G], *Msp*I-morph 4 in the gene for threonine tRNA [m.15925C>T], and *Nci*I-morph 2 in the *ND5* gene [m.13365C>T]) were associated with $\dot{V}O_{2max}$ at baseline, and one RFLP (*Hind*II-morph 1 in the *ND5* gene [m.12406G>A]) was associated with $\dot{V}O_{2max}$ response to 20-week training. The first study regarding the association of mtDNA polymorphisms with elite athlete status was reported in 1998. Rivera et al. (1998) examined the association between elite endurance athlete status and four mtDNA RFLPs, which as previously reported by Dionne et al. (1991), was associated with high $\dot{V}O_{2max}$ in the untrained state and its trainability. As a result, they found that there were no significant differences in frequencies of these polymorphisms between elite endurance athletes and sedentary controls (Rivera et al., 1998). In the next decade, two studies were conducted to examine the association between mtDNA polymorphism and $\dot{V}O_{2max}$ in

elite cyclists (Brearley, 2001) and sedentary males (Murakami et al., 2002). Murakami et al. (2002) sequenced the control region of mtDNA and reported that three (m.16298T>C, 16325T>C, and m.199T>C) and two (m.16223C>T and m.16362T>C) polymorphisms in the control region were associated with $\dot{V}O_{2max}$ at baseline and its response to 8-week endurance training, respectively. Furthermore, muscle biopsy samples obtained from the vastus lateralis muscle revealed that m.194C>T and m.514CA repeat polymorphisms were associated with citrate synthase (CS) activity and mtDNA content in human skeletal muscle, respectively. In addition, m.16519T>C polymorphism was significantly associated with the change rate of $\dot{V}O_{2max}$ and CS activity in response to training. The control region contains the origin of replication for the H strand, the promoters for H- and L-strand transcription, and the binding site of mitochondrial transcription factor A (TFAM) (Fernandez-Silva et al., 2003). Therefore, it is possible that these polymorphisms, located in the control region, were associated with $\dot{V}O_{2max}$ due to changes in the efficiency of transcription or replication of mtDNA. It was a substantively important study that showed associations of mtDNA polymorphisms with CS activity and mtDNA content in human skeletal muscle.

6.4 Mitochondrial haplogroup and elite athlete status

6.4.1 Mitochondrial haplogroup

A haplotype is a combination of alleles at multiple loci that are inherited together from a single parent, and a haplogroup is a group that shares similar haplotypes. The mtDNA can be categorized into mitochondrial haplogroups that are defined by the presence of a characteristic cluster of tightly linked mtDNA polymorphisms. Mitochondrial haplogroup distributions display geographic diversity. In African populations, haplogroups L0, L1, L2, and L3 are mainly observed. Among them, haplogroup L3 is proposed to be the ancestor of all non-African populations, and is a common root of macrohaplogroups M and N. European haplogroups, such as H, I, J, K, S, T, U, V, W, belong to macrohaplogroup N, whereas Asian haplogroups belong to both N and M macrohaplogroups (haplogroups A, B, F, and N9 belong to macrohaplogroup N, and haplogroups M7a, M7b, M8, D, and G belong to macrohaplogroup M). These regional differences in mitochondrial haplogroups are thought to be the results of natural selection and one of the important factors in the ancient adaptation of our ancestors to various environmental conditions, such as cold climates and/or famine (Mishmar et al., 2003). The functional differences between the most common European haplogroup H and African haplogroup L have been demonstrated by using cybrid cells, which are cell lines with identical nuclei, but contain mitochondria from different individuals with mitochondrial haplogroup H or L (Kenney et al., 2014). Cybrid cells with haplogroup L showed high expression levels of mtDNA-encoded respiratory complex genes, decreased ATP turnover rates, and low levels of reactive

oxygen species (ROS) production, despite lower mtDNA copy number. These results suggest that haplogroup L is associated with efficient OXPHOS systems. Furthermore, they found significant differences in the expression levels of nuclear DNA-encoded genes, which were unrelated to energy metabolism. The functional differences between mitochondrial haplogroups are considered to contribute to the differences in susceptibility of various diseases between ethnic populations.

6.4.2 Mitochondrial haplogroups and elite endurance athlete status

Eight studies have focused on the association of mitochondrial haplogroups with elite endurance athlete status (Table 6.1) since the first report of the association by Niemi et al. in 2005 (Niemi and Majamaa, 2005), who determined the mitochondrial haplogroup frequencies in Finnish elite endurance ($n=52$) and sprint/power ($n=89$) athletes and found significantly different frequencies of mitochondrial haplogroups. Elite endurance athletes showed lower frequencies of haplogroup K and haplogroup J compared with elite sprint/power athletes and controls, where none of the elite endurance athletes belongs to haplogroup K and subhaplogroup J2. This result was partially consistent with the previous association between haplogroup J and low $\dot{V}O_{2max}$ (Marcuello et al., 2009). Furthermore, the negative association between haplogroup K and elite endurance athlete status was replicated in the Polish populations (Maruszak et al., 2014). Subhaplogroup J2 and haplogroup K have previously been associated with longevity (De Benedictis et al., 1999; Niemi et al., 2003; Ross et al., 2001). Therefore, these haplogroups have opposite associations with elite endurance performance and longevity. Based on these findings, subhaplogroup J2 and haplogroup K are considered uncoupling genotypes. Elite endurance athletes should be a highly selected group in terms of efficiency of ATP production by OXPHOS. Mitochondrial OXPHOS produce ATP, while the uncoupling of the OXPHOS generates heat, which concomitantly reduces the production of ATP due to decreased proton translocation across the mitochondrial inner membrane or due to proton leak via ATP synthase (Kadenbach, 2003). Uncoupling of OXPHOS also reduces the production of ROS associated with aging. It was demonstrated that mice with deficient uncoupling produce more ROS (Hagen and Vidal-Puig, 2002) and mitochondria with enhanced uncoupling produce decreased ROS (Keipert et al., 2010). Interestingly, mice with enhanced mitochondrial uncoupling live longer (Speakman et al., 2004; Keipert et al., 2011). Therefore, an mtDNA genotype leading to less efficient OXPHOS and lower ATP production (an uncoupling genotype) would produce less ROS and probably promote longevity but could be a hindrance in endurance performance (Niemi and Majamaa, 2005).

Maruszak et al. (2014) examined the association between mitochondrial haplogroup and athlete status in elite Polish athletes and reported that Olympic/World-class endurance athletes exhibited a higher frequency of haplogroup H compared with Olympic/World-class sprint/power athletes. Haplogroup H has been reported to show higher $\dot{V}O_{2max}$ than haplogroup J, and mtDNA oxidative damage in skeletal muscle was higher

Table 6.1 Mitochondrial haplogroups associated with elite endurance athlete status

Reference	Participants	Ethnicity	Endurance-related haplogroups
European			
Niemi and Majamaa (2005)	52 elite endurance athletes (endurance runners and walkers) 89 elite sprint/power athletes (sprinters and field event athletes)	Finnish	Unfavourable: K, J (J2)
Castro et al. (2007)	95 elite endurance athletes (cyclists, endurance runners, and long-distance rowers) 250 controls	Spanish	Unfavourable: T
Nogales-Gadea et al. (2011)	102 elite endurance athletes (road cyclists, and endurance runners) 51 elite sprint/power athletes (jumpers, throwers, and sprinters) 478 controls	Spanish	V
Maruszak et al. (2014)	210 elite endurance athletes (athletes from various sports) 180 elite sprint/power athletes (sprinters, swimmers, and speed skaters) 400 controls	Polish	H Unfavourable: K (in men)
African			
Scott et al. (2005)	76 elite endurance athletes (endurance runners) 108 controls	Ethiopian	None
Scott et al. (2009)	291 elite endurance athletes (endurance runners) 85 controls	Kenyan	L0, M Unfavourable: L3
Asian			
Mikami et al. (2011)	79 elite endurance/middle-power athletes (athletes from various sports) 60 elite sprint/power athletes (athletes from various sports) 672 controls	Japanese	G
Kim et al. (2012)	75 elite endurance/middle-power athletes (athletes from various sports) 77 elite sprint/power athletes (athletes from various sports) 265 controls	Korean	M*, N9 Unfavourable: B

in Haplogroup H compared with haplogroup J (Martinez-Redondo et al., 2010). These results suggest that haplogroup H has a more coupling genotype compared with haplogroup J. Moreover, in European populations, two studies examined associations between elite Spanish endurance athlete status and mitochondrial haplogroups, but they reported conflicting results (Castro et al., 2007; Nogales-Gadea et al., 2011). The existing evidence

indicates that European haplogroups J and K are negatively associated, and haplogroup H is positively associated with elite endurance athlete status/performance.

In East African populations, which have been successful in international distance running (Wilber and Pitsiladis, 2012), the relationship of mitochondrial haplogroups with elite endurance athlete status have been examined. Scott et al. reported that mitochondrial haplogroups were associated with elite Kenyan athlete status (Scott et al., 2009), but not with elite Ethiopian athlete status (Scott et al., 2005). International Kenyan endurance runners displayed an excess of haplogroup L0 and a death of haplogroup L3 compared with the general Kenyan population, whereas national Kenyan endurance runners displayed an excess of haplogroup M. Although the study on Ethiopian endurance runners revealed that there was no significant association between the mitochondrial haplogroups and elite endurance athlete status, the elite Ethiopian endurance runners showed lower frequency of haplogroup L3 compared with the general Ethiopian population. Haplogroup L3, whose frequency was low in the elite Kenyan endurance athlete group, is considered a root of European and Asian haplogroups as mentioned above. Therefore, the presence or absence of polymorphisms determining haplogroups L3 possibly contribute to the success of East African populations in international distance running. It was shown that the frequency of haplogroup L0 associated with elite Kenyan athlete status is higher in East African populations compared with West African populations (Salas et al., 2004). In the recent Olympic Games and World Championships, the successes of individuals with West African ancestry stand out in the sprint event, while the successes of those with East African ancestry stand out in distance running. These geographical correlations with sports event characteristics may be affected by the regional differences of haplogroup distributions.

In Asian populations, two studies examined the association between mitochondrial haplogroups and elite endurance athlete status. In the Japanese population, the frequencies of mitochondrial haplogroups found in Olympic athletes from various sports were compared with those in the nonathletic Japanese controls (Mikami et al., 2011), and endurance/middle-power athletes displayed an excess of haplogroup G compared to controls. In addition, the frequency of haplogroup B in endurance/middle-power athletes tended to be lower than in controls. Kim et al. reported that in Korean Olympic athletes, endurance/middle-power athletes showed higher frequencies of haplogroups M and N9a, but a lower frequency of haplogroup B compared to nonathletic controls (Kim et al., 2012). Although a dearth of haplogroup B in endurance/middle-power athletes were observed in both Japanese and Korean Olympians, the other associations were not consistent between the two populations. As the effects of genotypes on the phenotypes are modified by environmental factors, it is possible that differences in environmental factors between two countries contribute to the inconsistency. These studies included various sports athletes in endurance/middle-power athlete groups. This heterogeneity of athlete group may influence the discrepancy of the results between studies.

6.4.3 Mitochondrial haplogroups and elite sprint/power athlete status

As sprint/power performance relies more on ATP produced by anaerobic glycolysis than by OXPHOS, the significance of mitochondrial function in sprint/power performance has been largely ignored. However, several studies have found significant associations between mitochondrial haplogroups and elite sprint/power athlete status (Table 6.2).

In the Japanese population, the frequency of mitochondrial haplogroup F was significantly higher in Olympians competing in sprint/power events than in nonathletic controls (Mikami et al., 2011). This association of haplogroup F with sprint/power athlete status was replicated in Japanese track & field athletes (Miyamoto-Mikami et al., 2017). Haplogroup F is a major component of the macrohaplogroup N, which was associated with higher leg extension power and vertical jump performance in nonathletic Japanese individuals (Fuku et al., 2012). Haplogroup F has previously been reported to be associated with the prevalence of type 2 diabetes mellitus (T2DM), while haplogroups N9a was associated with resistance against T2DM in Japanese and Korean populations (Fuku et al., 2007). Based on these findings, Hwang et al. (2011) conducted functional studies using cybrid cells and reported that cybrid cells harboring haplogroups F and N9a exhibited significant differences in their nuclear gene expression pattern; mitochondrial haplogroup F showed a decreased gene expression of mitochondrial OXPHOS pathway and an increased gene expression of the cytosolic glycolysis pathway compared with mitochondrial haplogroup N9a (Hwang et al., 2011). This observation can be regarded as a compensatory response for decreased ATP production caused by a defective mitochondrial haplogroup, resulting in an increased expression of nuclear genes involved in glycolysis. This phenomenon might explain, at least partly, the association between mitochondrial haplogroup F and elite sprint/power athlete status.

On the other hand, Deason et al. (2012) reported the association between mitochondrial haplogroup and elite athlete status in sprinters of sub-Saharan ancestry (Jamaican and African-American sprinters) (Deason et al., 2012). In that study, there was no significant difference in haplogroup frequencies between elite Jamaican sprinters and Jamaican controls. However, elite African-American sprinters showed higher frequency in non-African haplogroups, which included all haplogroups not commonly found in sub-Saharan Africa (also included haplogroup F), compared with African-American controls. This result suggests that the maternal admixture may play a role in sprint performance in African ancestry. Genomic data of 354,224 individuals from 102 cohorts showed that increased homozygosity was associated with decreased trait value, such as height, respiratory function, and general cognitive ability (Joshi et al., 2015). Therefore, it is possible that the more distant parental relatedness positively affect various traits, including exercise performance. Although the associations of mitochondrial haplogroups with elite sprint/power athlete status suggest the possibility that mtDNA variations could affect not only endurance, but also sprint/power performance, detailed underlying mechanisms remain unknown. Functional studies are required to investigate the underlying mechanisms of these associations.

Table 6.2 Mitochondrial haplogroups associated with elite sprint/power athlete status

Reference	Participants	Ethnicity	Sprint/power-related haplogroups
European			
Niemi and Majamaa (2005)	52 elite endurance athletes (endurance runners and walkers) 89 elite sprint/power athletes (sprinters and field event athletes)	Finnish	K, J
Nogales-Gadea et al. (2011)	102 elite endurance athletes (road cyclists, and endurance runners) 51 elite sprint/power athletes (jumpers, throwers, and sprinters) 478 controls	Spanish	None
Maruszak et al. (2014)	210 elite endurance athletes (athletes from various sports) 180 elite sprint/power athletes (sprinters, swimmers, and speed skaters) 400 controls	Polish	Unfavourable: H
African			
Deason et al. (2012)	107 elite Jamaican sprint/power athletes (sprinters and jumpers) 293 Jamaican controls	Jamaican	None
	119 elite African-American sprint/power athletes (sprinters and jumpers) 1148 African-American controls	African-American	Non-sub-Saharan haplogroups
Asian			
Mikami et al. (2011)	79 elite endurance/middle-power athletes (athletes from various sports) 60 elite sprint/power athletes (athletes from various sports) 672 controls	Japanese	F
Kim et al. (2012)	75 elite endurance/middle-power athletes (athletes from various sports) 77 elite sprint/power athletes (athletes from various sports) 265 controls	Korean	None
Miyamoto-Mikami et al. (2017)	211 sprint/power athletes (sprinters and field event athletes) 649 controls	Japanese	F

6.5 Analysis of entire mtDNA in elite athletes

The associations of mitochondrial haplogroups with elite athlete status may suggest that these haplogroups contain mtDNA variants that influence some aspect of athletic performance and/or its trainability. However, the association analysis of haplogroups does not identify the causal mtDNA variants. Therefore, to identify mtDNA variants that are associated with elite athlete status, we analyzed the entire mtDNA sequences (16,569 bp) of 185 elite Japanese athletes who had represented Japan at international competitions (Mikami et al., 2013). Sequence analysis of entire mtDNA of 185 elite Japanese athletes and 672 control subjects with various phenotypes, whose entire mtDNA sequences were registered in Human Mitochondrial Genome Single Nucleotide Polymorphism Database, detected a total of 1488 nucleotide variants. Among these variants, those with a minor allele frequency of equal to or higher than 1% in control group were defined as polymorphisms, while those with a minor allele frequency of lower than 1% in controls were defined as rare variants. Consequently, we detected 311 polymorphisms and 1177 rare variants in mtDNA of all participants. From the case-control association analysis of detected mtDNA polymorphisms, we found that 7 polymorphisms, namely, m.152T>C, m.514$(CA)_n$, poly-C stretch at m.568-573, m.4343A>G, m.11215C>T, m.15518C>T, and m.15874A>G are associated with elite endurance/middle-power athlete status. The frequencies of these seven polymorphisms were higher in the elite endurance/middle-power athletes than in the controls. Regarding m.514$(CA)_n$ repeat polymorphism, a total of six $(CA)_n$ repeat alleles were observed in the study population, ranging from three to eight repeats. When these alleles were divided into two groups, m.514$(CA)_{n\leq 4}$ and m.514$(CA)_{n\geq 5}$, a lower frequency of m.514$(CA)_{n\leq 4}$ alleles and higher frequency of m.514$(CA)_{n\geq 5}$ alleles were found in elite endurance/middle-power athletes compared to controls. Interestingly, Murakami et al. reported that the mtDNA content in the vastus lateralis muscle was higher in the healthy sedentary Japanese population with m.514$(CA)_5$ than those with m.514$(CA)_4$ (Murakami et al., 2002). It was reported that mtDNA content in the vastus lateralis muscle is closely correlated with CS activity and $\dot{V}O_{2\text{peak}}$ in healthy subjects (Wang et al., 1999). Hence, the increase of mtDNA content in the skeletal muscle of long m.514$(CA)_n$ repeat allele ($n \geq 5$) carriers could explain the association between m.514$(CA)_{n\geq 5}$ alleles and elite endurance/middle-power athlete status.

Another endurance/middle-power athlete status-related polymorphism, namely, m.4343A>G polymorphism, is located at the TψC loop region of the tRNA for glutamine. The tRNAs have a cloverleaf secondary structure due to four base-paired stems. This cloverleaf structure comprises three non-base-paired loops: D, anticodon, and TψC loops. Pathogenic mutations are often located at stem structures and tend to disrupt Watson-Crick nucleotide paring in the stem. The tRNA encoded by mtDNA is essential

for the synthesis of the 13 proteins of mitochondrial OXPHOS. Therefore, it is possible that this polymorphism could influence OXPHOS through improved efficiency in protein synthesis within the mitochondria. Recently, Wone et al. reported that the number of adenine repeats in tRNA for the arginine gene region varied significantly between mice selectively bred for 54 generations for high voluntary wheel running and mice randomly bred for 54 generations (Wone et al., 2019). The adenine repeat polymorphism exists in the D loop of the tRNA arginine and is associated with OXPHOS performance (Moreno-Loshuertos et al., 2006). These findings indicate the possibility that nucleotide variations in tRNA genes affect endurance performance.

On the other hand, analysis of the mtDNA sequences showed that 12 polymorphisms, namely, m.151C>T, m.204T>C, m.4833A>G, m.5108T>C, m.5601C>T, m.7600G>A, m.9377A>G, m.13563A>G, m.14200T>C, m.14569G>A, m.15314G>A, and m.16278C>T are associated with the elite sprint/power athlete status. Among them, three polymorphisms (m.151C>T, m.204T>C, and m.16278C>T) are located in the control region, and two polymorphisms cause amino acid replacements in ND2 (m.4833A>G: Thr122Ala) and Cytb (m.15314A>G: Ala190Thr). Although the impact of these polymorphisms is unknown, it is possible that these polymorphisms influence the transcription and replication of mtDNA and OXPHOS, respectively. In addition, the numbers of rare variants in the regions of the 12S rRNA and the ND1 genes were significantly higher in the sprint/power athletes than in the controls. It has been argued that a higher number of rare variants in certain genes could influence the susceptibility of Alzheimer's disease (Elson et al., 2006; Tanaka et al., 2010) and hypertriglyceridemia (Johansen et al., 2010), and these rare variants were predicted to have functional effects. Thus, these rare variants in the regions of the 12S rRNA and ND1 may influence elite athletic performance solely and/or through clustering.

In the last two decades, it has become clear that mitochondria-derived peptides (MDPs) are encoded by functional short open reading frames in the mtDNA, such as humanin, mitochondrial open reading frame of the 12S rRNA-c (MOTS-c), and small humanin-like peptides (SHLPs). Among them, a 16-amino-acid peptide named MOTS-c is encoded within the 12S rRNA and regulates insulin sensitivity and metabolic homeostasis (Lee et al., 2015). Recently, MOTS-c has been demonstrated to translocate to the nucleus and regulate nuclear gene expression following metabolic stress (Kim et al., 2018). The MOTS-c encoding region contains m.1382A>C polymorphism, which is specific for the Northeast Asian population. The m.1382A>C polymorphism causes lysine to glutamine replacement at 14th amino acid of MOTS-c and is associated with exceptional longevity (Fuku et al., 2015). Although we could not find any association between the m.1382A>C polymorphism and elite Japanese athlete status, we found an excess of rare variants in the 12S rRNA gene in elite sprint/power athletes. Therefore, it is possible that these rare variants influence the function or expression of MOTS-c. In addition to MOTS-c, other MDPs, such as humanin, and SHLP 1-6 have also been

shown to have several metabolic effects in various tissues (Kim et al., 2017). These findings suggest that mtDNA polymorphisms could influence not only mitochondrial function but also various cellular functions through these MDPs. To date, only one study has reported the whole mtDNA sequences in elite athletes (Mikami et al., 2013), and there are several limitations in that study, such as heterogeneity of athlete cohort, and the problem of multiple comparisons. Therefore, to conclude the role of mtDNA variants on elite athlete status, comprehensive analyses of mtDNA variants in more homogeneous elite athlete populations, replication studies, and functional studies are necessary.

6.6 Summary

Existing literature has demonstrated the association of mtDNA variants/haplogroups with elite athlete status. However, because these studies have not considered nuclear DNA variants, it is possible that these mtDNA variants/haplogroups are surrogates for nuclear DNA variants that confer the elite athletic performance [hitch-hiking effect (Bilal et al., 2008)]. Therefore, we need to consider these associations between mtDNA variants/haplogroups and elite athlete status with caution. To confirm the direct effects of mtDNA variants, functional studies using cybrid cells with identical nuclear DNA but different mtDNA, are important. In the field of sports science, genome-wide analysis such as the genome-wide association study (GWAS) has been introduced (Ahmetov et al., 2015; Rankinen et al., 2016). However, mtDNA variants have often been ignored in the analyses in spite of the importance of mtDNA in mitochondrial function and exercise performance. The influences of the interplay of the mtDNA and nuclear DNA on various phenotypes are clear (Latorre-Pellicer et al., 2016); therefore, concurrent genome-wide analyses (mitochondrial genome and nuclear genome) of elite athletes are required. Thus, consideration of the interactions between mtDNA and nuclear DNA variants will contribute to the elucidation of genetic factors of elite athletic performance.

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CHAPTER SEVEN

Psychogenetics and sport

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7.1 Introduction

In parallel with the burgeoning literature on expertise more widely (Hambrick et al., 2018), there has been an explosion of research into understanding the development of world-class athletic talent, with numerous models/frameworks forwarded (e.g., Long-Term Athlete Development: Balyi an d Hamilton, 2000; Developmental Model of Sport Participation: Côté et al., 2012; Dynamics of Talent Development: Davids et al., 2013; Differentiated Model of Giftedness and Talent: Gagné, 2015). Researchers generally agree that development relies on an interplay of aspects of the performer, the environment, and practice and training (Rees et al., 2016). At the same time, talent identification in sport has largely moved away from amore restricted focus on physiology and anthropometry, to the recognition of psychology as a key determinant of talent development (Blijlevens et al., 2018; MacNamara et al., 2010; Rees et al., 2016). However, very little research has considered whether the psychological traits of athletes have genetic origins, despite progress in understanding the genetics of physical prowess (Ahmetov et al., 2016), and earlier calls for such research (Lippi et al., 2008; Singer and Janelle, 1999). This is surprising, given that many traits which might be considered important for sport have been observed to show genetic inheritance in nonsport samples (e.g., personality factors are at least moderately heritable—see Rimfeld et al., 2016; as much as 50% of intelligence—the ability to learn, reason and solve problems—may be heritable—see Plomin and Deary, 2015). Table 7.1 lists the heritability estimates (from nonsport samples) for a number of traits which might also be important for sport.

Table 7.2 lists a number of the qualities (e.g., of memory, attention, decision time, reaction time, stress resistance, emotionality) that might plausibly be important for specific types of sports.

Sports, Exercise, and Nutritional Genomics
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Table 7.1 Heritability of several cognitive and personality traits.

Phenotype	Heritability (%)	References
Cognitive ability	48–50	Vogler et al. (2014) and Croston et al. (2015)
General memory	23–46	Reynolds and Finkel (2015)
Spatial ability	40–62	Reynolds and Finkel (2015)
Working memory	67	Croston et al. (2015)
Speed of perception of information	44–62	Croston et al. (2015)
Affective response (voluntary activity in sports)	82 (late adolescence)	Huppertz et al. (2012) and Schutte et al. (2017)
Mean reaction time	60	Kuntsi et al. (2006)
Temperament	30–60	Zwir et al. (2018)
Character	50–58	Zwir et al., (2018)
Aggression	49–65	Gelhorn et al. (2005), Burt et al. (2009), and Tuvblad and Baker (2011)
Novelty seeking	35–60	Zuckerman et al. (1993), Keller et al. (2005), and Fairbanks et al. (2011)
Harm avoidance	53	Keller et al. (2005)
Reward dependence	56	Keller et al. (2005)
Persistence	55	Keller et al. (2005)
Attention	28–38	Stins et al. (2005), Ocklenburg et al. (2016), and Xu et al. (2015)
Openness	49	Bae et al. (2013)
Conscientiousness	30–43	Givens et al. (2009) and Bae et al. (2013)
Extraversion	32–57	Keller et al. (2005), Givens et al. (2009), and Bae et al. (2013)
Agreeableness	18	Bae et al. (2013)
Neuroticism	25–54	Keller et al. (2005), Givens et al. (2009), and Bae et al. (2013)

Table 7.2 Specific characteristics of cognitive and personality traits in athletes.

Group	Psychological characteristics	References
Endurance athletes	Introversion, calmness, plasticity of mental processes, strategic and tactical thinking, low emotionality, sense of purpose, stress resistance, low scores of anxiety, high level of motivation, sense of time and pace, hardiness, ability to distribute power over time, low scores of neuroticism, great life satisfaction	Bäckmand et al. (2001), Egloff and Gruhn (1996), Ilyin (2016), and Yurov (2013)
Game sports athletes	High emotional intelligence, communicability, high level of motivation, mobility of the nervous system, reaction speed, emotional control, responsibility, sanguine	Pozdnyshev (2014), Ilyin (2016), Szabo and Urbán (2014)

Table 7.2 Specific characteristics of cognitive and personality traits in athletes—cont'd

Group	Psychological characteristics	References
Combat athletes	Analytical and operational thinking, self-discipline, high volume of attention and attention-sharing, self-trust, a tendency to self-expression, neurotic	Bäckmand et al. (2001), Mäkelä (1974), Ilyin (2016), and Costarelli and Stamou (2009)
Strength and speed-strength (jumpers, throwers) athletes	Assertiveness, operational thinking, self-control, emotionality, ability to mobilize quickly, persistence, speed reaction, attention set-shifting, leadership, strong type of temperament, extraversion, state of being organized, sociability, often sanguine, self-containment, mental endurance, risk-taking	Pozdnyshev (2014), Bäckmand et al. (2001), and Ilyin (2016)
Sprinters	Confidence, operative thinking, self-control, emotionality, ability to mobilize quickly, mobility of excitation and inhibition, persistence, attentional set-shifting, leadership, extraversion, self-discipline, emotional stability, high level of competitive motivation, risk-taking	Ilyin (2016) and Yurov (2013)

7.2 Psychogenetic markers and sport

The assumption of sports psychogenetics is that the polymorphism of a given gene may affect the cognitive (e.g., memory, type of thinking, reaction/response time, and attention) and personality (e.g., aggression, motivation, and type of temperament) traits of an athlete. Understanding how best to search for polymorphisms of candidate genes that might map onto the psychology of expert athletes requires knowledge of the molecular mechanisms of the central and peripheral nervous systems.

Genetic markers that favor the development of such psychological qualities as resistance to stress, attention, reaction time, adaptation to change, do in one way or another, involve the neurotransmitter system. In particular, various stimulating and motivational aspects of behavior are associated with the serotonergic and dopaminergic systems.

Case-control studies remain the most common study design in sports psychogenetics and generally involve determining whether one allele of a DNA sequence (gene or non-coding region of DNA) is more common in a group of elite athletes than it is in the

general population. For example, Peplonska et al. (2019) by comparing allelic frequencies between 621 athletes and 672 sedentary controls have identified 7 potential psychogenetic markers (*FEV* rs860573, *SLC6A3* rs6347, *SLC6A2* rs2242446, *HTR1B* rs11568817, *TPH2* rs7305115, *NR3C2* rs2070951, *HTR2C* rs3813929) associated with endurance, power, and combat athlete statuses. Cross-sectional (genotype-phenotype) association studies are another type of study design in sports psychogenetics and examine whether athletes with one genotype (or allele) of a particular DNA sequence show different measures of a trait (e.g., reaction time, self-confidence, etc.) compared to the rest of the sample. To date, 16 genetic markers have been reported to be associated with predisposition to specific sports (via case-control designs) (Table 7.3), and 12 markers have been linked with personality traits (via genotype-phenotype designs) (Table 7.4).

Table 7.3 Psychogenetic markers discovered in case-control association studies involving athletes.

Gene, SNP	Participants	Favorable allele	Phenotype	References
AVPR1A	85 Dancer (classical ballet, modern dance, jazz ballet), 872 controls, 91 athletes (runners, swimmers, basketball, and volleyball players)	RS1, RS3	Dancer athlete status	Bachner-Melman et al. (2005)
ACE I/D	189 Athletes engaged in endurance sports (cross-country skiing and rowing), synchronized swimming, and ice-hockey players vs 212 volunteers	D	Swimming athlete status	Shleptsova et al. (2008)
BDNF rs6265	55 Marathon runners and endurance bicyclists vs 58 controls	C (Val)	Endurance athlete status	Haslacher et al. (2015a)
COMT rs4680	51 Ultra-endurance Ironman athletes vs 56 recreationally active controls	Met (A)	Endurance athlete status	van Breda et al. (2015)
	364 Healthy men vs 199 combat athletes (boxing ($n = 98$), karate ($n = 82$) and different martial arts ($n = 19$)	A	Combat athlete status	Leźnicka et al. (2018)
DAT VNTR	50 Elite athletes (Caucasian, Afro-American, Afro-European and Maori ethnicities): soccer ($n = 4$), basketball ($n = 10$), tennis ($n = 6$), volleyball ($n = 6$), canoeing ($n = 2$), rugby ($n = 10$), baseball ($n = 6$), and track and field (speed running, javelin and shot put) ($n = 6$) vs 100 healthy controls practicing sport activity at lower levels	9 repeat	Mix cohort athlete status (game sports)	Filonzi et al. (2015)

Table 7.3 Psychogenetic markers discovered in case-control association studies involving athletes—cont'd

Gene, SNP	Participants	Favorable allele	Phenotype	References
	214 Men convicted of crimes of different gravity, 425 subjects of control group, 107 MMA fighters	9 repeat, 10 repeat	Combat athlete status	Cherepkova et al. (2017)
DRD4 48-bp VNTR	425 Controls, 107 MMA fighters	7 repeat	MMA fighters athlete status	Cherepkova et al. (2017)
	Female subjects: 106 subjects of control group, 62 young professional synchronized swimmers, 41 older athletes—synchronized swimmers, track-and-field athletes, cyclists, and rowers; Male: 40 subjects of control group, 28 athletes doing combat sports (Sambo wrestlers)	S	Synchronized swimmers status, combat sports status	Sysoeva et al. (2010)
SLC6A4 (5-HTTLPR)	62 Caucasian female synchronized swimmers, 104 Caucasian female volunteers	LL	Synchronized swimmers status	Sysoeva et al. (2009)
	85 Dancer (classical ballet, modern dance, jazz ballet), 872 participants of control group, 91 athletes (endurance athletes: runners, swimmers, basketball, and volleyball players)	S +12 VNTR	Dancer status	Bachner-Melman et al. (2005)
	223 Male athletes (endurance sports), 177 male nonathletes	L	Endurance athlete status	Trushkin et al. (2011)
5HT1A rs6295	63 Marathon runners and endurance bicyclists vs a control group of 73 participants	C	Endurance athlete status	Haslacher et al. (2015a)
OPRM1 rs1799971	364 Healthy men vs 199 combat athletes (boxing ($n = 98$), karate ($n = 82$) (minimum level 1 Kyu) and different martial arts ($n = 19$)	G	Combat athlete status	Leźnicka et al. (2018)
*FEV*rs860573	621 Elite athletes (212 endurance, 183 power and 226 combat athletes) vs 672 sedentary controls	A	Power, endurance and combat athlete status	Peplonska et al. (2019)
SLC6A3 rs6347	621 Elite athletes (212 endurance, 183 power, and 226 combat athletes) vs 672 sedentary controls	C	Power and combat athlete status	Peplonska et al. (2019)
SLC6A2 rs2242446	621 Elite athletes (212 endurance, 183 power, and 226 combat athletes) vs 672 sedentary controls	T	Combat athlete status	Peplonska et al. (2019)

Continued

Table 7.3 Psychogenetic markers discovered in case-control association studies involving athletes—cont'd

Gene, SNP	Participants	Favorable allele	Phenotype	References
HTR1B rs11568817	621 Elite athletes (212 endurance, 183 power, and 226 combat athletes) vs 672 sedentary controls	C	Combat athlete status	Peplonska et al. (2019)
TPH2 rs7305115	621 Elite athletes (212 endurance, 183 power, and 226 combat athletes) vs 672 sedentary controls	G	Endurance athlete status	Peplonska et al. (2019)
NR3C2 rs2070951	621 Elite athletes (212 endurance, 183 power, and 226 combat athletes) vs 672 sedentary controls	G	Endurance athlete status	Peplonska et al. (2019)
HTR2C rs3813929	621 Elite athletes (212 endurance, 183 power, and 226 combat athletes) vs 672 sedentary controls	T	Power athlete status (by women only)	Peplonska et al. (2019)

Table 7.4 Psychogenetic markers discovered in genotype-phenotype association studies involving athletes.

Gene, SNP	Participants	Favorable allele	Phenotype	References
ACE I/D	87 Tunisia athletes (100, 200, and 400 m sprints, 110 and 400 m hurdles, long, and triple jump, 5000 and 10,000-m long-distance running, 10-km race walks)	I	Self-confidence (less scores anxiety)	Znazen et al. (2016)
APOE ε4	53 Football players	ε3, ε2	Cognitive performance	Kutner et al. (2000)
	250 Student-athletes (95 football, 38 baseball, 35 soccer, 32 soccer, 20 softball, 16 basketball, 2 basketball, 1 cross-country, 1 track and field)	ε3, ε2	Reaction time	Cochrane et al. (2018)
BDNF rs6265	141 High-risk athletes (skydiving, free ride skiing, mountaineering, rock climbing, mountain biking and paragliding)	C (Val)	High-risk sport athletes status	Thomson et al. (2015)

Table 7.4 Psychogenetic markers discovered in genotype-phenotype association studies involving athletes—cont'd

Gene, SNP	Participants	Favorable allele	Phenotype	References
	and 132 low-risk athletes (running, cycling, swimming, weight lifting, yoga, triathlon, golf and cross-country skiing)			
COMT rs4680	250 Student-athletes (95 football, 38 baseball, 35 soccer, 32 soccer, 20 softball, 16 basketball, 2 basketball, 1 cross-country, 1 track and field)	Met	Impulse control scores	Cochrane et al. (2018)
	1119 Elite athletes (marathon)	Val	Warrier, low anxiety	Sims et al. (2018)
	57 Asian male competitive swimmers (23 freestyle swimmers, 5 backstroke swimmers, 10 breaststroke swimmers, 11 butterfly swimmers, and 8 individual medley swimmers)	Met	Competitive performance of competitive swimmers	Abe et al. (2018)
	303 Apparently healthy male rugby players	Met	Anticipatory worry	Mc Fie et al. (2018)
	146 Athletes of different specializations and qualifications	Met	Psychological stability	Valeeva et al. (2019)
DRD2 rs1076560	72 Young healthy females of Caucasian/ European descents (nonathletes)	G	Performance in the sequence motor learning task	Noohi et al. (2013)
DRD3 rs167771	671 Skiers and snowboarders	A	Sensation seeking	Thomson et al. (2013a, b)
DRD4 rs1800955	503 Skiers and snowboarders	C	Sensation seeking	Thomson et al. (2013a, b)
MAOA VNTR	13 Athletes of combat sports	Short allele	Rigidity, motivation for success	Kalaev et al. (2015)
	24 Basketball players	L	Successful in sports	Ulucan et al. (2014)

Continued

Table 7.4 Psychogenetic markers discovered in genotype-phenotype association studies involving athletes—cont'd

Gene, SNP	Participants	Favorable allele	Phenotype	References
	133 Elite athletes (soccer, basketball, hockey)	L	Cognitive anxiety, emotional arousal control, emotional aspects, neuroticism, tension-anxiety	Petito et al. (2016)
	180 Athletes (football and hockey players)	L	Indirect aggressiveness	Guba and Marinich (2016)
	86 Synchronized swimmers (women)	L	A sense of perception of time, indirect aggressiveness	Maliuchenko et al. (2007)
5-HTTLPR **SLC6A4* rs25531	303 Apparently healthy male rugby players	Low (S_A/S_A) and intermediate (S_A/L_A, S_A/L_G, L_A/L_G, L_G/L_G)	Low harm avoidance, anticipatory worry and fear of uncertainly; high novelty seeking	Mc Fie et al. (2018)
5HT2A rs6313	86 Synchronized swimmers	C	A faster flow of time	Maliuchenko et al. (2007)
STMN1 rs182455	141 High-risk athletes (skydiving, free ride skiing, mountaineering, rock climbing, mountain biking and paragliding) and 132 low-risk athletes (running, cycling, swimming, weight lifting, yoga, triathlon, golf, and cross-country skiing)	T	High-risk sport athletes status	Thomson et al. (2015)
STMN1 rs213641	141 High-risk athletes (skydiving, free ride skiing, mountaineering, rock climbing, mountain biking and paragliding) and 132 low-risk athletes (running, cycling, swimming, weight lifting, yoga, triathlon, golf, and cross-country skiing)	G	High-risk sport athletes status	Thomson et al. (2015)

7.2.1 Serotonergic system

The serotonergic system is one of the key neurotransmitter systems in the brain. A wide range of receptor subtypes of this system, common in most cortical and subcortical structures, is indicated in influencing behavior and psychopathic states. Serotonin (also known as 5-hydroxytryptamine or 5-HT) plays a key role in early developmental periods, acting as a growth factor, and regulating the development of its own and related neural systems (Whitaker-Azmitia et al., 1995; Bonnin et al., 2007). It also acts as a neurotransmitter in the mature brain, regulating cognition, attention, emotion, pain, sleep, and arousal. By using molecular genetics to study the metabolism of the serotonergic system, candidate genes of human emotional properties can be determined. Serotonin receptors are grouped into 7 families, which include 15 genes (Hoyer et al., 1994). It would be interesting to examine whether polymorphisms of the genes of the serotonergic system are related to athlete status and indeed whether they differentiate athletes from different types of sports.

Gene *SLC6A4* is encoded by the human gene of the serotonin transporter (solute carrier family 6 member 4; localization: 17q11.1-q12) and is expressed in the central and peripheral nervous systems and platelets (Lesch et al., 1993). The serotonergic system is involved in the regulation of mood and emotional characteristics, affecting aggressiveness aimed at oneself and others (Hessl and Tassone, 2008; Linnoila et al., 1983; Sysoeva et al., 2009). The *SLC6A4*-linked polymorphic region (5-HTTLPR) is a degenerate repeat polymorphic region in *SLC6A4*. The polymorphism is found in the promoter region of the *SLC6A4* gene. Researchers have noted two variations of this gene polymorphism: L (long; 44-bp insertion)—and S allele (short; deletion) (Heils et al., 1996; Nakamura et al., 2000). 5-HTTLPR is associated with an altered response of the serotonin system. When the 5-HTT is present, the short allele shows a decreased gene expression and fewer serotonin transporters in the membrane of the cell (Caspi et al., 2003; Curran et al., 2005). In turn, this leads to the short allele of 5-HTTLPR functioning as an emotion amplifier, which may confer heightened susceptibility to environmental conditions (Haase et al., 2015), predicting heightened stress reactivity (Caspi et al., 2010), heightened negative emotional reactivity (Gyurak et al., 2013; Hariri et al., 2002), as well as heightened self-conscious emotional reactivity (Gyurak et al., 2013), and low levels of openness in adults (Rahman et al., 2017).

Some studies have shown better working memory scores for homozygous LL (Zilles et al., 2012; Havranek et al., 2015), whereas SS genotype carriers perform poorer in memory updating (Weiss et al., 2014). Consistent with the role of 5-HT in coping with stress, the S-allele has been associated with increased hypothalamic-pituitary-adrenal-axis stress-reactivity (Miller et al., 2013), and greater risk for developing stress-related affective pathology (Jans et al., 2007; Karg et al., 2011; Capello and Markus, 2014). Experimental data with athletes have shown that the low-active 5-HTT polymorphism

(SS genotype) may be associated with scores on covert aggression, indirect hostility, and negativism in synchronized swimmers (Sysoeva et al., 2009). The latter study observed a higher frequency of LL genotype in synchronized swimmers when compared with a control group (53% vs 33%) (Sysoeva et al., 2009). A study by Trushkin and colleagues similarly found a higher incidence of the LL genotype in male endurance athletes (rowers, skiers, cyclists, biathletes) compared to a reference group (39 vs 25%) (Trushkin et al., 2011). The assumption from this work is that L carriers have a greater resistance to stress, because the L-allele has higher transcriptional efficiency, resulting in increased serotonin binding, mRNA expression, and serotonin availability (Heils et al., 1996; Lesch et al., 1996).

It would be interesting to consider the combination of variable number tandem repeat (VNTR) with 5-HTTLPR polymorphism variants in the SLC6A4 gene. The VNTR region is found in intron 2 (STin2) has 9, 10, and 12 repeat units, which consist of 16–17 bp repetitive elements (Furlong et al., 1998). Levels of enhancer activity vary based on the allele, with each repeat unit making varying contributions to activity. VNTR can connect and cooperate with the 5-HTTLPR regulatory element. VNTR polymorphisms are associated with a variety of behavioral and stress-related states in combination with other polymorphisms (such as the 5-HTTLPR). A study by Bachner-Melman and colleagues with dancers found an association between the short HTTLPR promoter region and 12 repeat VNTR and high scores on the *Absorption Scale* of *Tellegen's* Multidimensional *Personality* Questionnaire (Tellegen and Atkinson, 1974; Bachner-Melman et al., 2005). The same authors also considered the effect of *AVPR1A* gene (arginine vasopressin receptor 1a; located on 12q14.2) on dance performance. The two-locus RS1 and RS3 haplotypes were also significantly associated with the *Absorption Scale* (Bachner-Melman et al., 2005). *AVPR1* has also been associated with the Reward Dependence subscale of the Tridimensional Personality Questionnaire, and may also be involved in human social communication, because the evolutionary basis of vasopressin plays a key role in courtship behavior that frequently involves elements of song and dance (Williams, 2001).

The 5-hydroxytryptamine receptor 1A (*5HT1A*) gene is located on chromosome 5 (localization: 5q12.3). In the central nervous system, the receptor subtype 5-HT1A is expressed in the cerebral cortex, hippocampus, septum, amygdala, and other structures of the limbic system, in the raphe nucleus on the soma and dendrites of 5-HT neurons or postsynaptic receptors, basal ganglia and thalamus (Ito et al., 1999; De Almeida and Mengod, 2008; Filip and Bader, 2009; Buhot et al., 2000). This gene is known for its functional polymorphism rs6295, which is located in the promoter region and which regulates HTR1A transcription and region-specific modification of HTR1A expression (Wu and Comings, 1999; Albert and Lemonde, 2004). Specifically, the G-allele of the rs6295 leads to higher expression of the 5-HT1A gene (Gong et al., 2014), which leads to an increase in 5-HT1A autoreceptors and decrease in postsynaptic 5-HT1A receptor levels (Albert et al., 2011a,b). The polymorphism rs6295 thus may explain

the risk of developing mental illness. As noted in the work of Haslacher et al. (2015b), the C-allele may protect against depressive mood in elderly endurance athletes (risk of development in the control group of 30% vs 2% of athletes of the C-carrier allele).

Given that athletes' cognitive functioning and psychological skills are key factors in their ability to navigate a successful career in sport, it is necessary to understand the involvement of polymorphisms of the serotonergic system genes (Wrisberg and Anshel, 1989; Holm et al., 1996; Vaeyens et al., 2007). Serotonin receptor 1A is involved in memory and plays a key role in learning. It is located at high concentration levels in the hippocampus as well as in the raphe nucleus (Buhot et al., 2000). CC-carriers have also been shown to have better working and episodic memory (Yen et al., 2014; Wesnes et al., 2016; Long et al., 2017).

7.2.2 Dopaminergic system

The dopaminergic system is a reward system that operates in the consolidation of memory for learning, motor skills, and executive function. Dopamine is a key neurotransmitter in the central nervous system involved in reward-related incentive learning (Pessiglione et al., 2006, 2007). Dopamine receptors are part of a G-protein-coupled receptor family that also includes D1, D2, D3, D4, and D5. Genes of the dopaminergic system may be involved in the traits of athletes. The following outlines polymorphisms associated with the dopamine system that have been noted in case-control studies in sport.

The dopamine receptor D_4 (*DRD4*) gene is located near the telomere on 11p15.5, which contains 4 exons. The D4 subtype is a G-protein coupled receptor which inhibits adenylyl cyclase. This dopamine receptor is responsible for neuronal signaling in the mesolimbic system of the brain, an area of the brain which regulates emotion and complex behavior. Mutations in this gene have been associated with various behavioral phenotypes, including risk taking (Mallard et al., 2016; McGeary et al., 2007; Shao et al., 2006; Abidin et al., 2015). The *DRD4* contains several polymorphic loci in the promoter region. For example, the C allele of the rs1800955 polymorphism has been shown to be associated with novelty seeking and extraversion. Another polymorphism is a variation of a 48 base pair tandem repeat (VNTR) in exon 3, ranging from 2-repeat (2R) to 11-repeat alleles (11R) (Chang et al., 1996). Humans with the 7R allele show increased levels of physical activity (Faraone et al., 2001; Kluger et al., 2002; Grady et al., 2003, 2005, 2013; Li et al., 2006) and are more responsive to external factors (Sheese et al., 2007; Belsky et al., 2009; Olsson et al., 2013; Grady et al., 2013). The 7R allele is associated with financial risk-taking (Dreber et al., 2009; Muda et al., 2018), attention-deficit/hyperactivity disorder (Faraone et al., 2001; Grady et al., 2003, 2005), alcoholism (MacKillop et al., 2007), and disinhibition (Congdon et al., 2008). In a study with Mixed Martial Arts fighters, athletes were shown to have an increased frequency of 7R alleles compared with controls (Cherepkova et al., 2017).

The *DRD2* gene encodes the D2 subtype of the dopamine receptor. The gene is located on 11q23.2. Thompson et al. (1997) found an association between theTaq1 polymorphism of the *DRD2/ANKK1* gene and individual differences in dependency and impulsivity in nonathletic participants (Thompson et al., 1997). Although Abe et al. (2018) showed no differences in the frequency of alleles between competitive swimmers and controls, Noohi et al. (2013) identified an association between the *DRD2* rs1076560 G allele and performance in a sequence motor learning task in young healthy (albeit nonathlete) females of Caucasian/European descent.

The *DRD3* gene is located on 3q13.3. This receptor is localized to the limbic areas of the brain, which are associated with cognitive, emotional, and endocrine functions (Sokoloff et al., 1990). Variation in this gene may be associated with susceptibility to hereditary essential tremor. Abe et al. (2018) could not establish a link between the *DRD3* rs6280 polymorphism and swimmers' competitive performance. However, in another study, Thomson et al. (2013a,b) found an association between a *DRD3* variant (rs167771) and the sensation-seeking of skiers and snowboarders.

7.3 Conclusion

There is evidence that elite athlete development has a genetic basis. Our current progress toward understanding the molecular basis of the cognitive abilities and personality traits of athletes is, however, in its infancy. The next decade may well be an exciting period for sports psychogenetics, with the application of new DNA technologies (e.g., whole genome sequencing; GWAS; epigenomic, transcriptomic, and proteomic profiling) and bioinformatics to dissect and analyze genetic effects on behavior of athletes. This chapter provides preliminary evidence that at least 16 genetic markers have been reported to be associated with predisposition to specific sports (via case-control designs), and 12 markers have been linked with personality traits (via genotype-phenotype designs). However, it should be emphasized that there is wide variability in designs and sample types, and results have yet to be replicated in independent samples. It should be noted that each DNA locus may explain only a very small proportion of phenotypic variance (e.g., ∼0.1% to ∼1%), such that both very large sample sizes, and combinations of gene loci, may be needed to detect associations of interest. Since DNA polymorphisms for cognitive abilities and personality traits of athletes do not fully explain the known heritability of such traits, other forms of variation, such as rare mutations and epigenetic markers (i.e., stable and heritable changes in gene expression), must also be considered. The issues with respect to appropriate study designs, sample size, population stratification, and quality of genotype/phenotype measurement are also of great importance. Future genetic research with large cohorts of athletes, with further validation and replication, will substantially contribute to the discovery of causal genetic variants (i.e., mutations and DNA polymorphisms) that may partly explain the heritability of athlete status and related psychological phenotypes.

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SECTION II

Exercise genetics and molecular physiology

CHAPTER EIGHT

Genes and response to aerobic training

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8.1 Introduction

Skeletal muscle is the most abundant tissue in mammals and primarily supports their movements, but it is also involved in the whole-body metabolism regulation (Baskin et al., 2015). It is characterized by different metabolic traits reflecting myofiber composition. Regular exercise has several effects on human body such as increases endurance capacity and muscle strength, improves arterial stiffness, maintains body weight, reduces risk of chronic diseases (Booth et al., 2012) is protective for insulin resistance (Bruce et al., 2006; Castaneda et al., 2002; Goodpaster et al., 2001; Zanuso et al., 2010), modulates lipid metabolism (Watt and Hoy, 2012), and contrasts age-related decline in enzyme activity or protein content (Menshikova et al., 2006).

The process of exercise-induced adaptation in skeletal muscle involves a multitude of signaling mechanism initiating replication of specific DNA genetic sequences, enabling subsequent translation of the genetic message, and ultimately generating a series of amino acids that form new proteins. The functional consequences of these adaptations are determined by training volume, intensity and frequency, and the half-life of the proteins (Coffey and Hawley, 2007). It should be noted that at the molecular and cellular levels individuals with the same genotype respond more similarly to training than those with different genotypes, indicating that genes play an important role in determination of individual differences in response to training. The search for genetic markers of trainability status will likely be more productive than the investigation of molecular markers of the performance phenotype in the untrained state (Bouchard et al., 1997).

Prolonged endurance training elicits a variety of metabolic and morphological changes, including mitochondrial biogenesis, fast-to-slow fiber-type transformation, and substrate metabolism. Endurance adaptation results in increased muscle glycogen stores and glycogen sparing at submaximal lactate kinetics and morphological alterations, including greater type I fiber proportions per muscle area, and increased capillary and

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mitochondrial density. Repeated bouts of endurance exercise result in altered expression of a multiplicity of gene products, resulting in an altered muscle phenotype with improved resistance to fatigue (Adhihetty et al., 2003). In addition, heart rate (HR) and blood pressure (BP) recovery acceleration after exercise are also important cardiovascular adaptations to endurance training. For instance, postexercise HR recovery improves after endurance training in sedentary healthy subjects and patients with cardiovascular diseases, however, a substantial heritable component may also be involved in the regulation of HR behavior in response to training, and this may partly explain the large interindividual variation in HR recovery. Several studies reported the associations of gene polymorphisms with postexercise HR and BP recovery (reviewed in Bray et al., 2009).

The effects from exercise differ greatly among individuals, depending on lifestyle factors and genetic backgrounds. From studies on identical twins, it is known that heredity plays a significant role in determining the interindividual variability to several stimuli (Bouchard and Tremblay, 1997; Haworth et al., 2008; Polderman et al., 2015). At the begun of 1990 the HERITAGE training Family Study was planned to understand the role of the genotype in the response to aerobic exercise to cardiovascular and metabolic diseases. The research involved different laboratories in United States and Canada to recruit about 700 healthy sedentary subjects (white and blacks) that were tested for different variables (oxygen uptake, BP, HR, blood lactate, maximal oxygen uptake, etc.) before and after 20 weeks of exercise. Within subjects in the study it was included parents and biological offspring that were used to understand the association between genetic variance and the variability in responsiveness to training.

Several other projects were developed to understand genomics and transcriptomics of athletic performance and responses to training: GELAK, GELAV, GUAP, ELITE, GAMES, GENESIS, Gene SMART, GOING, J-HAP, NTR, POWERGENE, Super-Athletes, 1000 Athlome all reviewed in Wang et al. (2016). In 2015, a group of main investigators in sports and exercise science reviewed the main findings in exercise genetics and genomics during a symposium held in Santorini. They agree that sport genomics have to shift from the analysis of candidate genes to unbiased exploration of the genome; for this reason it was lunched a collaborative initiative: the Athlome Project Consortium (Pitsiladis et al., 2016).

The HERITAGE study evidenced that there was an extreme interindividual variability in the training response. The average increase in VO_{2max} was 19%, but about 5% of subjects demonstrated little or no change in VO_{2max} and 5% a high increase (40%–50%). The variance was higher between families than within families for the gains in VO_{2max}, but there was no relationship with the initial VO_{2max} and its increase. This allows inferring that there is a group of genes responsible for VO_{2max} and a different group responsible for its increasing after the training. The maximal heritability estimate of the VO_{2max} response to training adjusted for age and sex was reported to be 47% (Bouchard et al., 1999).

Genetically gifted athletes have a much greater response to training. Accordingly, evidence that many of the world's best endurance runners originate from distinct regions of Ethiopia and Kenya, rather than being evenly distributed throughout their respective countries appears to further sustain the idea that the success of East African runners is genetically mediated (Scott et al., 2003). Studies have shown that African distance runners have reduced lactic acid accumulation in muscles, increased resistance to fatigue, and increased oxidative enzyme activity, which equates with high levels of aerobic energy production. It has been proposed that these geographical disparities in athlete production may reflect a genetic similarity among those populating these regions for an athletic genotype and phenotype (Scott and Pitsiladis, 2006).

An understanding of genetic backgrounds will help to clarify criteria of daily physical activities and appropriate exercise for individuals including athletes, making it possible to apply individualized preventive medicine and medical care. Moreover, health benefit will result from enhanced health, physical functioning, and lifestyle improvement (Maeda et al., 2006).

8.1.1 Gene variants: Association with responses to aerobic exercise

One hundred and one DNA polymorphisms have been reported to be associated with interindividual responsiveness to aerobic training (Ahmetov and Rogozkin, 2009; Xu et al., 2016; Williams et al., 2017). Most recent review considered 35 studies to understand variants associated with VO_{2max} trainability (Williams et al., 2017). This study reported 98 DNA variations as predictors for the contribution of VO_{2max} trainability. The majority of considered studies were based on cross-sectional retrospective designs basing the identification of genes associated with VO_{2max} trainability on genome-wide association studies (GWAS) or on the analysis of candidate genes. Fourteen (*ACE* Alu I/D, *ACSL1* rs6552828, *AMPD1* rs17602729, *APOE* rs7412 and rs429358, *CAMTA1* rs884736, *CD44* rs353625, *CKM* rs8111989, *DAAM1* rs1956197, *NDN* rs824205, *Near BIRC7* rs6090314, *RGS18* rs10921078, *RYR2* rs7531957, *ZIC4* rs11715829) of the 98 predictor genetic variations were replicated between or within at least two studies. In addition, there are three genetic markers (*ACE* Alu I, *AMPD1* rs17602729 C, *GSTP1* rs1695 G) that are associated with both VO_{2max} trainability and endurance athlete status (Ahmetov and Fedotovskaya, 2015). In total this makes 15 markers which are less likely false-positive than others (Table 8.1). Of those, four genetic markers, as the most significant, are briefly described below.

Adenosine monophosphate deaminase (AMPD) is an important regulator of muscle energy metabolism by converting AMP to inosine monophosphate (IMP) and therefore favoring the synthesis of ATP (2 ADP ↔ ATP + AMP). AMPD expression is influenced by physical activity (Gineviciene et al., 2014). The nonsense mutation c.34C > T (C to T transition in nucleotide 34, rs17602729) of the *AMPD1* gene converts glutamine codon (CAA) into the premature stop codon (TAA) causing the premature interruption

Table 8.1 Genetic variances associated with VO_{2max} trainability

Gene	Location	Polymorphism	Favorable allele for VO_{2max} training response	References
ACE	17q23.3 intron	Alu I/D	I	Hagberg et al. (1998), Defoor et al. (2006), and Ahmetov et al. (2008)
ACSL1	4q35.1 intron	rs6552828	G	Bouchard et al. (2011) and Ghosh et al. (2013)
AMPD1	1p13.2 exon (nonsense)	rs17602729	C	Rico-Sanz et al. (2003) and Thomaes et al. (2011)
APOE	19q13.32 exon (missense)	rs7412 rs429358	T (ε2) C (ε4)	Thompson et al. (2004) and Yu et al. (2014)
CAMTA1	1p36.31 intron	rs884736	G	Bouchard et al. (2011) and Ghosh et al. (2013)
CD44	11p13 intron	rs353625	TBC	Bouchard et al. (2011) and Ghosh et al. (2013)
CKM	19q13.32 3′UTR	rs8111989	A	Rivera et al. (1997) and Rivera et al. (1999)
DAAM1	14q23.1 intron	rs1956197	G	Bouchard et al. (2011)
GSTP1	11q13.2 exon (missense)	rs1695	G	Zarebska et al. (2014, 2017)
NDN	15q11.2 genomic region 5′ upstream the gene	rs824205	A	Bouchard et al. (2011)
Near BIRC7	20q13.33 genomic region 5′ upstream the genes	rs6090314	A	Bouchard et al. (2011) and Ghosh et al. (2013)
RGS18	1q31.2 genomic region 5′ upstream the gene	rs10921078	A	Bouchard et al. (2011)
RYR2	1q43 intron	rs7531957	TBC	Bouchard et al. (2011) and Ghosh et al. (2013)
ZIC4	3q24 intron	rs11715829	G	Bouchard et al. (2011)

The table includes variants associated with VO_{2max} trainability confirmed in at least two studies. TBC, to be confirmed whether variant contributes to a high or low training response. Genomic information is based on GRCh38/hg38.
Modified from Williams, C.J., Williams, M.G., Eynon, N., Ashton, K.J., Little, J.P., Wisloff, U., Coombes, J.S., 2017. Genes to predict VO2max trainability: a systematic review. BMC Genomics 18, 831.

of protein synthesis and AMPD deficiency (Gineviciene et al., 2014). Part of the population expressing the mutant AMPD1 T allele (2% of the Caucasian population are homozygous [TT genotype] and approximately 20% are heterozygous [CT genotype]) are vulnerable to muscular cramps, pain, and premature fatigue during exercises (Gineviciene et al., 2014; Kar and Pearson, 1981). In a study of Rico-Sanz et al. (2003), subjects with the AMPD1 XX genotype had diminished exercise capacity and cardiorespiratory responses to exercise in the sedentary state. In a study of 935 Coronary

artery disease patients the carriers of the X allele had a significantly lower relative increase in peakVO_2 after 3 months of aerobic training (Thomaes et al., 2011).

The creatine kinase muscle (*CKM*) gene codes for a protein responsible for the transfer of phosphate between ATP and various phosphogens such as creatine phosphate. The rs8111989 A/G *CKM* gene polymorphism in the 3′UTR was shown to be associated with physical performance. In a study of 160 Caucasian parents and 80 adult offspring of the HERITAGE Family Study, the aerobic performance was associated with *CKM* genotype (Rivera et al., 1997). VO_{2max} was measured during cycle ergometry tests before and after 20 weeks of endurance training. *CKM* genotype in parents was significantly associated with VO_{2max}. A significantly greater VO_{2max} response to endurance training program was detected in parents and offspring with *CKM* AA/AG genotypes compared to the GG genotype. In a following study, Rivera et al. (1999) have confirmed these results in 277 full sib pairs from 98 Caucasian families.

Apolipoprotein E (APOE) variants affect cell lipid uptake, the level of lipids in the blood, and endothelial vascular dilation (Leon et al., 2004). APOE has three common alleles: ε2, ε3, and ε4 constructed from two missense SNPs (rs7412, rs429358). VO_{2max} increased only 5% in the E3/E3 subjects versus 13% in the E2/E3 and E3/E4 groups in response to 6 month exercise training and these differences were significantly different among the genotypes (Thompson et al., 2004). Accordingly, Chinese men and women, 18–40 years old, with the E2/E3 and E3/E4 genotypes showed a significantly higher VO_{2max} training response compared to other APOE genotypes following 6 months of progressive moderate intensity continuous training (Yu et al., 2014).

Variances described in Table 8.1 are located in different gene parts: 5′-UTR, exon, intron, 3′-UTR. This is interesting from a functionally point of view, in fact, if it is normal to think that variants within exons can cause alterations in coded proteins (if nonsynonymous variants), variances in introns may produce new splicing sites (Kurmangaliyev et al., 2013), while alterations within the 5′-UTR may influence translational regulation by influencing mRNA stability and translation efficiency (Pickering and Willis, 2005) and within 3′-UTR regions may alter microRNA function (Moszynska et al., 2017).

It should be noted that there are more than 100 genetic markers that have been reported to be associated with endurance athlete status and baseline VO_{2max} (Ahmetov et al., 2009, 2015, 2016; Bray et al., 2009; Fedotovskaya et al., 2014; Mustafina et al., 2014; Rankinen et al., 2016), and these that should be tested for association with VO_{2max} trainability in the future.

8.1.2 Noncoding RNAs as modulators of exercise

Noncoding RNAs represent the major portion of RNA transcripts in the human and mouse genome (Kashi et al., 2016) and include short (18–25 nt long; microRNAs or miRNAs) and long noncoding RNAs (>200 nt long; lncRNAs).

MicroRNAs. miRNAs originate from a pre-miRNAs (kb long) processed in the nuclei by type III RNAses (DROSHA) to produce the pre-miRNAs (60–70 nt long) that are actively translocated in the cytoplasm where the activity of DICER, another RNAse, allows the generation of a double-stranded RNA of about 22 nt (Shukla et al., 2011). miRNAs regulate gene expression posttranscriptionally binding to specific sites in the 3′-UTR of their target mRNAs and causing transcript degradation or the block of protein synthesis (Shukla et al., 2011). There are also examples of miRNA regulatory activity by binding the 5′-UTR of their targets (Lee et al., 2009), but the first method based on 3′-UTR binding is the most studied. It is interesting that each miRNA can have different targets and each gene can be targeted by different miRNAs because the target recognition is not based on perfect base pairing. miRNAs can have a restricted pattern of expression and this feature served to identify MyomiRs, miRNAs that are enriched or specifically expressed in the striated muscles. We evidenced that MyomiRs can have a different expression pattern in different myofiber types (Chemello et al., 2019) showing the importance of a single myofiber approach to dissect pathway regulation in a such heterogenous tissue as skeletal muscle. MyomiRs are listed in Table 8.2 in association with their prevalent expression in different myofiber types.

Several miRNAs respond to exercise in skeletal muscle or heart (Table 8.3). In this chapter we not discuss extensively resistance exercise. We discuss in detail results of aerobic training that, contrarily to resistance exercise, does not cause evident changes in

Table 8.2 MyomiRs

	Expression value in myofibers			
miRNA	**Oxidative**	**Glycolytic**	**Expression pattern**	**Representative literature**
miR-1	>16	>16	Heart, skeletal muscle	Chen et al. (2006, 2010) and Horak et al. (2016)
miR-133a	>14	>14	Heart, skeletal muscle	Deng et al. (2011), Horak et al. (2016), and Yin et al. (2013)
miR-206	>13	Not expressed	Skeletal muscle	Horak et al. (2016), Soares et al. (2014), and Williams et al. (2009)
miR-208a	N/A	N/A	Heart	Horak et al. (2016) and van Rooij et al. (2007, 2009)
miR-208b	>10	Not expressed	Heart, skeletal muscle	Horak et al. (2016) and van Rooij et al. (2009)
miR-486	>12	>12	Heart, skeletal muscle	Horak et al. (2016)
miR-499	N/A	N/A	Heart, skeletal muscle	Horak et al. (2016) and van Rooij et al. (2009)

In the table are listed all MyomiRs in association with representative literature and expression value in myofibers obtained by next-generation sequencing experiments. Expression values are Log2 of sequencing results normalized as counts of reads per million (+1). *N/A*, not available data.

Table 8.3 Alteration of miRNA expression after exercise

Type of exercise	Species	miRNA	Outcome	Representative literature
Chronic overload	Mouse	miR-1, -133a ↓	Hypertrophy	Kirby and McCarthy (2013) and McCarthy and Esser (2007)
Acute resistant exercise	Human	miR-1 ↓	Hypertrophy	Drummond et al. (2008) and Kirby and McCarthy (2013)
Chronic resistant exercise	Human	miR-378 ↓ ↑ miR-451	Low response to resistant exercise	Davidsen et al. (2011) and Kirby and McCarthy (2013)
Acute running	Mouse	miR-23 ↓ ↑ miR-1, -181, -107	Mitochondrial biogenesis	Kirby and McCarthy (2013) and Safdar et al. (2009)
Chronic running	Mouse	miR-696 ↓	Mitochondrial biogenesis	Aoi et al. (2010) and Kirby and McCarthy (2013)
Acute cycling	Human	↑ miR-1, -133	Modulate response to training	Kirby and McCarthy (2013) and Nielsen et al. (2010)
Chronic cycling	Human	miR-1, -133a, -133b, -206 ↓	Modulate response to training	Kirby and McCarthy (2013) and Nielsen et al. (2010)
Chronic cycling	Human	miR-1, -101, -133, -455 ↓	Angiogenesis	Keller et al. (2011) and Kirby and McCarthy (2013)
Acute swimming	Mouse	miR-494 ↓	Mitochondrial biogenesis	Kirby and McCarthy (2013) and Yamamoto et al. (2012)
Chronic swimming	Rat	miR-1, -133, -133b ↓ ↑ miR-29c	Increases compliance	Kirby and McCarthy (2013) and Soci et al. (2011)
Chronic swimming	Rat	↑ miR-129	Angiogenesis	Da Silva Jr et al. (2012) and Kirby and McCarthy (2013)
Chronic swimming	Rat	miR-143 ↓ ↑ miR-27a, -27b	Hypertrophy	Fernandes et al. (2012) and Kirby and McCarthy (2013)
Chronic swimming	Rat	miR-16 ↓	Angiogenesis	Fernandes et al. (2011) and Kirby and McCarthy (2013)

Down oriented arrows indicate the down regulation of miRNAs while the others the upregulation.

muscle mass but induces metabolic adaptations. The increase of muscle mass after resistance exercise is due to the upregulation of insulin growth factor 1 (IGF-1) and the activation of AKT pathway (Schiaffino and Mammucari, 2011). It is interesting that miR-1 and -133a, that target AKT pathway, are downregulated after resistance exercise. For a depth discussion of miRNAs altered during resistance exercise see D'Souza et al. (2017), Kirby and McCarthy (2013), and Zacharewicz et al. (2013).

During endurance exercise miRNAs respond accordingly to the duration of the stimulus. Early responders (in 3 h of stimulus) are miR-1, -133a, -133b, and -181a that are upregulated and miR-9, -23a, -23b, and -31 that were downregulated (Russell et al., 2013). Late responders (following 12 weeks or 10 days of training) are miR-1, -133a, -133b, -206, and -31 that were downregulated (Nielsen et al., 2010; Russell et al., 2013) and miR-29b that was upregulated (Russell et al., 2013). From the analyses performed by Nielsen et al. (2010) and Russell et al. (2013), it was evident that the timing of the analysis is important for determining miRNA expression. In fact, after 12 weeks of training miR-1 resulted downregulated while after 10 days it resulted upregulated. Timmons et al. evidenced that genes responsive to endurance training show an overrepresentation of runt-related transcription factor 1 (RUNX1), sex-determining region Y box-9 (SOX9), and paired box gene-3 (PAX3) transcription factor-binding sites within their promoter regions making these transcription factors potential modulators of muscle aerobic adaptation (Timmons et al., 2010). It is interesting that the analysis of the same subjects for the alteration of miRNA expression identified 21 miRNAs altered (Keller et al., 2011) with miR-92, -98, -101, and -104 that were predicted to target RUNX1, SOX9, and PAX3. This result suggests that the downregulation of these miRNAs during endurance exercise may allow the muscle aerobic adaptation. As previously reported, PGC-1α is the master regulator of mitochondrial biogenesis and the expression of the gene encoding for this protein (PPARGC1A) is regulated by miR-23 (Wang et al., 2015) and miR-696 (Aoi et al., 2010) that respond to prolonged aerobic exercise in mice (Safdar et al., 2009). Aerobic exercise down modulates also the expression of miR-494 that targets the mitochondrial transcription factor A (TFAM) a key activator of mitochondrial transcription (Yamamoto et al., 2012).

MicroRNAs can be secreted in the blood and can function for the communication among different sites in the body and different tissues. Moreover, they can be used as biomarkers for pathologies or physical fitness. In fact, plasma concentration of miR-146a and -20a predicts VO_{2max} and the trainability of VO_{2max}, respectively (Baggish et al., 2011). Also miR-21 and -210 are associated to the VO_{2max} with an inverse relationship (Bye et al., 2013) such as miR-486 (Aoi et al., 2013). These data support the opportunity to use secreted miRNAs, instead of using bioptic samples, to monitor exercise capacity and trainability of the people. It is important to stress the fact that cited miRNAs that are secreted after training are not specific for the response to exercise, but their secretion is altered also in cardiovascular diseases. Therefore, it should be more and more efforts to understand targets of secreted miRNAs during training, and their function. It is known beneficial effects of exercise in general health and in particular on heart and recently it was revised the opportunity that this action is regulated by secreted miRNAs (Wang et al., 2018).

Long noncoding RNAs. A relative recent class of noncoding RNAs was discussed for its importance in the skeletal muscle pathophysiology: the long noncoding RNAs

(lncRNAs). It was demonstrated that these lncRNAs are involved in different processes of the skeletal muscle (Ballarino et al., 2016; Goncalves and Armand, 2017; Hagan et al., 2017; Li et al., 2018; Lim et al., 2018). Recently, using a single-cell approach, we demonstrated that they are differentially expressed in different myofiber types and that the lncRNA Pvt1 impacts on the skeletal muscle atrophy (Alessio et al., 2019). LncRNAs function as an additional regulator of the genome via multiple mechanisms completely different from those used by miRNAs (Brosnan and Voinnet, 2009). LncRNAs modulate transcription initiation, elongation, and termination (Wang and Chang, 2011). In addition, lncRNAs are involved in the modulation of mRNA splicing, transport, translation, stability, and subcellular localization of proteins (Anko and Neugebauer, 2010; Gong and Maquat, 2011; Yoon et al., 2012). Moreover, they can influence miRNA activity by binding them and avoiding their action on their specific targets (Cesana et al., 2011). Research on lncRNAs in the skeletal muscle is in its infancy and there are not many works that associate their function with exercise. In the 2015 Anderson and colleagues showed that a putative lncRNA synthesized for a micropeptide (myoregulin; MLN) that colocalizing with the SERCA1, responsible for the calcium transport across the sarcoplasmic reticulum, regulates its activity. It is interesting that KO mice for MLN demonstrated improved exercise performances (Anderson et al. 2015). This result associate lncRNAs and skeletal muscle exercise opening a new way to molecularly explain interindividual, muscle specific, or myofiber specific responses to exercise.

8.1.3 A cross-link between long noncoding RNAs and health induced by endurance exercise

The end of linear eukaryotic chromosomes is protected from continuous shortening and rearrangements by telomeres. These are heterochromatic structures that in some cases are transcribed into a class of long noncoding RNAs, named telomeric repeat-containing RNAs (TERRA), with a transcription start site that is localized in subtelomeric regions. TERRA RNA can regulate telomere length modulating endonuclease 1 and telomerase, repair damaged telomeres recruiting chromatin modifiers, and modulates telomere protein composition during cell cycle (Azzalin and Lingner, 2015). Telomere length decreases during aging and thus it is considered as a biomarker of cellular senescence, oxidative stress, and aging. This is linked to various aging associated diseases such as diabetes, hypertension, Alzheimer's disease, and cancer (Rizvi et al., 2014; Sanders and Newman, 2013). To understand better the connection between regular exercise and benefit for a healthier life Diman and colleagues decided to measure if telomere length is influenced by exercise. The study involved 10 persons that ride with a cycle machine for 45 min. After that blood samples and muscle biopsies were collected to compare with those collected before the running step. Researchers found that running activity induced the transcription of TERRA in the skeletal muscle (Diman et al., 2016). In the same

work, Diman et al. showed that the transcription of TERRA lncRNA is influenced by nuclear respiratory factor 1 (NRF1) and AMPK/PGC-1α with the activation of AMPK that induces NRF1-dependent increase of TERRA. These results suggest that bicycle exercise (aerobic exercise) activate processes that allow telomeres lengthening to slowing the aging process.

8.1.4 Memory of skeletal muscle: The role of epigenetic modifications

With skeletal muscle memory we intend the capacity of skeletal muscle to respond differently to environmental stimuli in an adaptive or maladaptive manner if the stimuli have been previously encountered. These processes and several aspects associated with the transmission in newborn of metabolic traits of maternal caloric, nutrient, and protein restriction are well discussed in the review of Adam and colleagues (Sharples et al., 2016). Here we will consider aspects associated with exercise. Originally muscle memory was intended as the better ability of adult human skeletal muscle to respond more advantageously to stimuli that have already been encountered in the past. In "Gene variants: Association with responses to aerobic exercise" section we discussed the ability of exercise to modulate specific gene expression by modulating DNA methylation or histone methylation or acetylation. This is particularly useful in the "self-"treatment, but it is particularly interesting that an exercise stimulus in the parents can be 'remembered' by the offspring. This is an important message for the health of offspring that appear to be also under the control of specific attitudes of the parents. Laker et al. demonstrated that exercise in obese mice rescued hypermethylation of PGC-1α and corresponding reductions in mRNA levels of PGC-1α, Glut4, Cox4, and CytoC together with the loss in metabolic function in later life of the offspring (Laker et al., 2014). Moreover, Romero and colleagues evidenced that aerobic exercise training performed by parents reduce visceral offspring adiposity, the diameter of subcutaneous adipocytes, and improved metabolic parameters associated to metabolic syndrome (Romero et al., 2018). Recently, it was developed another interesting concept. Most people know that the diet and exercise habits of a pregnant woman impacts the health of her baby, but little is known about how a father's health choices are passed to his children. Stanford et al. demonstrated that voluntary exercise training of male mice results in pronounced improvements in the metabolic health of adult male and female offspring (Stanford et al., 2018). Which is the driver of this action? The authors of the scientific work analyzed small RNAs in the sperm of trained or untrained mice demonstrating marked change in the levels of multiple small RNAs with the potential to alter phenotypes in the next generation. One more time the noncoding component of the genome appears to be functionally determinant for the health and also for the muscle memory.

8.1.5 Pathways involved in the response of aerobic exercise (coding genes)

Analyses on single genes permitted to evidence that adenosine 5′-diphosphate (ADP)/adenosine 5′-triphosphate (ATP) ratio increases during exercise. This leads to the activation of adenosine 5′-monophosphate (AMP)-activated protein kinase (AMPK) and therefore to the phosphorylation of PGC-1α that translocate in the nucleus where, in association with other transcription factors (e.g., SIRT1, RXR, NRF1), regulates several genes (Lin et al., 2002; Oka et al., 2011; Puigserver et al., 2001). Other signals such as Ca^{2+} oscillations, oxidative stress, or reduction/oxidation state are related to the activation of PGC-1α (Camera et al., 2016) (Fig. 8.1). Transcriptomic (Keller et al., 2011; Neubauer et al., 2014; Timmons et al., 2010; Vissing and Schjerling, 2014) and proteomic (Burniston and Hoffman, 2011; Fiuza-Luces et al., 2018; Petriz et al., 2012) analyses were performed to understand genes regulated by endurance exercise. Gene ontology (GO) analysis of transcripts responding to endurance training evidenced that a large number of these genes is related to extracellular matrix and immune response (Keller et al., 2011;

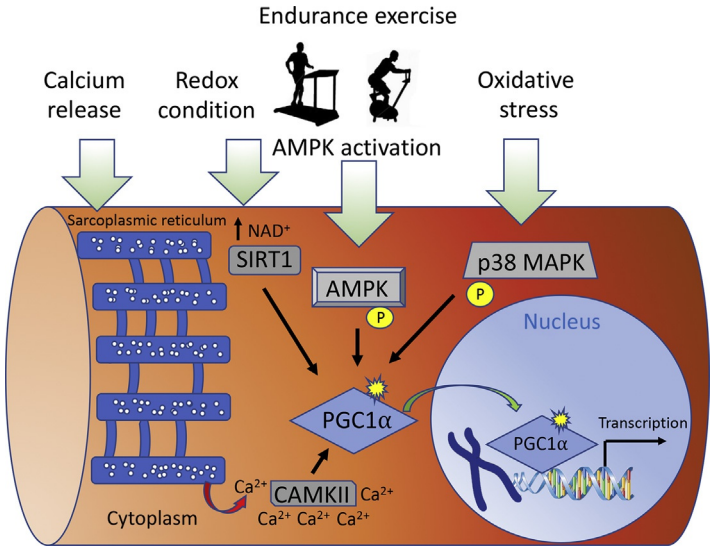

Fig. 8.1 Signals propagated by endurance-based exercise. Endurance exercise activates several kinases probably in response to stress generated. Calcium is an important mediator of muscle contraction and its release from sarcoplasmic reticulum activates Ca^{2+}-dependent protein kinase II; (CAMKII). The alteration of ADP/ATP ratio leads the activation of MP-dependent kinase (AMPK); oxidative stress induced by the activity is able to induce p38 mitogen-activated kinase (p38 MAPK), and, finally, the redox state may influence the activity of sirtuin 1 (SIRT1). SIRTs are deacetylase families NAD dependent that control histone acetylation. All cited modifications impinge on PGC-1α phosphorylation regulating its nuclear translocation and the activation of a transcriptional program for genes that create an oxidative phenotype.

Timmons et al., 2010). GO enrichment analysis groups genes according their function without considering the importance of their topology in a pathway. To better understand processes involved in specific conditions it may be useful to consider biological pathways. We tested if altered genes described in Timmons et al. (2010) significantly modulate specific pathways. It is interesting that pathways involved in integrin signaling, angiogenesis through vascular endothelial growth factor (VEGF), and those associated with inflammation mediated by chemokines and cytokines or T-cell activation are activated. There is a close link between endurance running and the activity of the immune system (Barros et al., 2017) and this was also sustained by these data. Also considering data described in Vissing and Schjerling (2014) and taking in account responses following exercise, angiogenesis appeared the most impacted mechanism. Therefore, aerobic exercise appeared to influence angiogenesis. This is also sustained in a recent work (Kwak et al., 2018). The ability of aerobic exercise of modulating angiogenesis in of particular interest to counteract phenomena related to aging and senescence where angiogenesis in inhibited and the expression of angiogenic factors as VEGF is decreased.

8.1.6 Aerobic exercise and diseases

People with chronic diseases such as heart disease, diabetes, asthma, back, or joint pain may benefit from exercise. In fact, regular exercise can help to manage symptoms of chronic diseases improving health. For example, people suffering of heart disease can improve their heart health with regular exercise and people with high BP can reduce their BP with exercise. Exercise is a cure-all for diabetes and can allow the controlling weight. Regular aerobic exercise can ameliorate back pain reinforcing abdominal and back muscles. Not only exercise improves the stability of junctions ameliorating arthritis, but also improves the quality of life for people who have a cancer, also improving their fitness (https://www.cancer.gov/about-cancer/causes-prevention/risk/obesity/physical-activity-fact-sheet). The risk of dying for breast, colorectal, and prostate cancer decrease in people that make regular exercise (Clague and Bernstein, 2012; Shi et al., 2017). Finally, exercises have an impact also on cognition in people. People who are active are at less risk of dementia and cognitive impairment (Mandolesi et al., 2018).

Which are the mechanisms that relate physical exercise to cancer and cognitive impairment? A number of plausible mechanisms have been proposed, some specific to one particular cancer. However, the single mechanism or group of mechanisms that explains the associations between physical activity and lower cancer risk has yet to be established (Clague and Bernstein, 2012). For colon cancer, the major hypothesis is that physical activity lowers fecal bile acid concentrations and decreases gastrointestinal transit time. For breast cancer, physical activity may alter the production of sex steroid hormones by altering menstrual cycle patterns (resulting in luteal phase defects, oligomenorrhea, or secondary amenorrhea) through its impact on the hypothalamic

pituitary axis and, among postmenopausal women, by controlling body weight (Anzuini et al., 2011). Other mechanisms with a more anticancer impact of physical activity include heightening immune surveillance, reducing inflammation, increasing endogenous antioxidant enzyme systems, increasing insulin sensitivity, controlling growth factor production and activation, decreasing obesity and central adiposity, optimizing DNA repair capacity, and reducing oxidative stress (Anzuini et al., 2011). It is plausible that most of these mechanisms act simultaneously and interact synergistically to mediate the associations between physical activity and cancer.

Dementia and cognitive impairment are associated to aging. Exercise may enhance cognition indirectly by improving health conditions (stress, sleep) and reducing chronic diseases (coronary heart diseases) that impact neurocognitive functions (Spirduso et al., 2005). Physical activity induces angiogenesis, as shown before, and neurogenesis with changes in molecular growth factors such as brain-derived neurotrophic factor (BDNF), which plays a crucial role in neuroplasticity and neuroprotection, and increased production of insulin-like growth factor 1 (IGF-1), which is involved in both neurogenesis and angiogenesis (Bherer et al., 2013; Erickson et al., 2011).

The major problem in understanding the benefit of physical activity is the uncontrolled and variability of conditions used in different studies (intensity of the exercise, duration). Future studies should be focused on the comprehension of the intensity, duration, and types of exercise that better enhance cognitive conditions and people health.

8.2 Conclusions

In this chapter, we considered different aspects of genomics, transcriptomics, and epigenomics of exercise adaptation in humans. This is a promising research field especially considering the possibility to associate a mechanism to alterations associated with muscle performances. Therefore, it is required that the causative mechanisms directly linking genotype, epigenetic alterations, and transcriptional profiles to phenotype are more clearly deciphered. The simple associations of genomic variants with phenotype is the starting point to better update practice especially considering the possibility of transgenerational transmission, as in the case demonstrated by Stanford et al. (2018).

It is important to remember that mechanisms altered by exercise are many and different and therefore considering the exercise at systemic level could be an improvement for the comprehension of its importance in the maintenance of people health. The integration of information about SNPs, epigenetic alterations, and gene expression may be the future for a better comprehension of mechanisms altered by exercise. It is also important to consider aspects related with the control of the exercise. In fact, different training programs impact differently on gene regulation.

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CHAPTER NINE

Effect of gene polymorphisms on sensitivity to resistance training

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9.1 Introduction

Resistance exercise training is widely used to enhance general fitness and athletic potential across many sporting disciplines, including power, strength, and endurance events (American College of Sports Medicine, 2009). When properly performed and combined with adequate nutrition and rest, resistance training can lead to increases in strength, power, speed, muscle size, local muscular endurance, coordination, and flexibility as well as reductions in body fat and blood pressure (Kraemer and Ratamess, 2004).

An effective resistance exercise program involves manipulation of several variables specific to the targeted goals, such as intensity or load per repetition [i.e., percentage of one repetition maximum (1 RM)], volume (total number of sets and repetitions), training frequency, muscle action (concentric vs eccentric), rest intervals between sets, repetition velocity, and other factors. Current guidelines for resistance training state that loads $\geq$65% one-repetition maximum (1 RM) are necessary to elicit significant increases in muscle hypertrophy (Kraemer et al., 2002). Higher loads are needed to maximize strength (American College of Sports Medicine, 2009; Kraemer and Ratamess, 2004). It has been postulated that heavy loading is required to fully recruit higher-threshold motor units. These data suggest that strength and muscle hypertrophy can be optimized via complete motor unit activation using heavy weight loads. However, a recent study suggested that low-intensity exercise, such as 30%–40% 1 RM, could induce a level of muscle hypertrophy similar to results from high-intensity resistance training (Schoenfeld et al., 2015; Mitchell et al., 2012). Mitchell et al. (2012) demonstrated an effect of resistance training with low loads (30% 1 RM) and high repetitions on muscle hypertrophy. The results suggested that there is a different optimal load and method for obtaining muscle hypertrophy between high- (70%–85% 1 RM) and low-load (30%–40% 1R M) resistance training.

Previous studies have been shown muscle strength and mass are heritable phenotypes, with a heritability range of 14%–80% for strength (Thomis et al., 1998b; Reed et al., 1991;

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Arden and Spector, 1997) and 20%–85% for muscle mass (Arden and Spector, 1997; Thomis et al., 1998b). A recent review using metaanalysis suggested that heritability of the strength and power phenotype is 49%–56% (Zempo et al., 2017). Although heritability adaptation of these muscle phenotypes to resistance training has not been well studied, the adaptive response also appears to have a genetic component. The results from a study of five pairs of monozygotic (MZ) twins submitted to a 10-week strength training program suggested that the response to strength training was independent of the genotype (Thibault et al., 1986). However, a significant genotype associated with training interactions was found for dynamic strength phenotypes in 25 pairs of MZ and 16 pairs of dizygotic (DZ) twins subjected to a 10-week strength training program for the elbow flexor (Thomis et al., 1998a). The F ratio was 3.5 for 1 RM increase with training, and 1.83 for maximal isometric contraction at 110 degrees response, indicating twin pairs responded similarly to strength training. Model-fitting procedures indicated that about 20% of the variation in posttraining 1 RM, isometric strength at 110 degrees, and concentric strength at 120 degrees/s was explained by training-specific factors, which were independent from genetic factors that explained variation in the baseline phenotype (Thomis et al., 1998a). Some previous studies reported an association between gene polymorphisms, such as single nucleotide polymorphisms (SNPs), and response to resistance training (Tables 9.1 and 9.2). Using genetic profiling to better match individual genotypes with an appropriate training modality may be a powerful tool to provide a more personalized, and precise resistance training prescription in the future. In this chapter, we summarize research on genetics and responses to resistance training.

9.2 Genetic markers associated with response to resistance training

9.2.1 ACE I/D genotype

Angiotensin-converting enzyme (ACE) is widely expressed in human tissues, including skeletal muscle, and may play a metabolic role during exercise (Jones and Woods, 2003). Moreover, the ACE insertion/deletion (I/D) polymorphism was the first genetic factor reported to influence human physical performance (Montgomery et al., 1998). The D allele is present at a higher frequency in power-oriented athletes (Kikuchi et al., 2012), whereas the I allele is overrepresented among elite endurance athletes (Woods et al., 2000). An I/D polymorphism in this gene has been found to be responsible for half of the variation in ACE enzyme activity (Rigat et al., 1990), with those who carry the deletion (D) allele having higher ACE enzyme activity (Danser et al., 1995). Homozygotes for the I allele (II) have significantly less ACE activity than heterozygotes (ID), and heterozygotes have lower ACE activity than homozygotes for the D allele (DD) in blood (Tiret et al., 1992) and muscle (Yan et al., 2018). The polymorphism corresponding to the insertion (allele-I) or deletion (allele-D) of the ACE gene at 287

Table 9.1 Genetic studies of baseline difference and responses to resistance training.

Study	Candidate gene	Polymorphism rs number	No. of subjects (% women)	Training status	Measurements	Baseline difference	Responses to training	Significant allele (type)
Thomis et al. (2004)	ACE	I/D	57 (0%)	Untrained	Strength, CSA	No	No	
Charbonneau et al. (2008)	ACE	I/D	243 (57%)	Untrained	MVC	Yes	No	DD genotype
Pescatello et al. (2006)	ACE	I/D	631 (58%)	Untrained	MVC	No	Yes	II/ID genotype
Pereira et al. (2013)	ACTN3	R577X	139 (100%)	Untrained	Maximal strength, power	No	Yes	R allele
	ACE	I/D				No	Yes	DD genotype
Erskine et al. (2014)	ACTN3	R577X	51 (0%)	Untrained	MV and 1RM	Yes	No	R allele
	ACE	I/D				No	No	
Gentil et al. (2011)	ACTN3	R577X	141 (0%)	Untrained	MT	No	Yes	R allele
Sood et al. (2012)	IGF-I	CA repeat	114 (54%)	Untrained	Power	Yes	No	192 heterozygotes and all other genotype
Hand et al. (2007)	IGF-I	CA repeat	128 (55%)	Untrained	Strength, MV	No	Yes	Interaction with PPP3R I/D genotype
	IGFBP3	A-202C				No	No	
	PPP3R1	I/D				No	Yes	II genotype
Kostek et al. (2005)	IGF-I	CA repeat	67 (52%)	Untrained	Strength, MV	No	Yes	192 allele
Hong et al. (2014)	CNTF	rs1800169	83 (0%)	Untrained		No	No	
Walsh et al. (2009)	CNTF	rs1800169	754 (60%)	Untrained	MVC	No	Yes	GG genotype
Li et al. (2014)	MSTN	A55T	94 (0%)	Untrained	MT	Yes	Yes	AT + TT geontype
		K135R				Yes	Yes	KR genotype

Continued

Table 9.1 Genetic studies of baseline difference and responses to resistance training—cont'd

Study	Candidate gene	Polymorphism rs number	No. of subjects (% women)	Training status	Measurements	Baseline difference	Responses to training	Significant allele (type)
Popadic Gacesa et al. (2012)	B2BRK	−9/+9	29 (0%)	Untrained	CSA	No	Yes	-9 allele
Erskine et al. (2012)	PTK2	rs7460	51 (0%)	Untrained	MVC	Yes	Yes	TT genotype
		rs7843014				No	Yes	AC + CC genotype
Harmon et al. (2010)	CCL2	rs17652343	874 (59%)	Untrained	Strength	No	No	
		rs1860189				No	No	
		rs3917878				No	No	
		rs2857654				No	No	
		rs1024611				Yes	No	AA genotype
		rs1024610				No	Yes	AT/TT genotype
		rs3760396				No	No	
		rs2857656				Yes	No	GC/CC genotype
		rs2857657				No	No	
		rs4586				Yes	No	CT/CC genotype
		rs13900				Yes	No	CT/TT genotype
	CCR2	rs17141010				No	No	
		rs768539				Yes	No	CC genotype
		rs3918358				No	Yes	AA genotype
		rs1799864				No	No	
		rs1799865				No	Yes	TT genotype
Riechman et al. (2004)	IL-15RA	rs3136617	153 (50%)	Untrained	LBM	No	Yes	BstNI/HpaII
		rs3136618				No	Yes	haplotype
		rs2296135				No	Yes	AA genotype
Pistilli et al. (2008)	IL-15	rs1057972	748 (60%)	Untrained	Strength, MV	No	Yes	T allele
		rs1589241				No	No	
	IL-15RA	rs3136618				No	No	
		rs2228059				Yes	No	AA genotype
		rs2296135				No	Yes	C allele

CSA, cross-sectional area; *MVC*, maximal voluntary contraction; *MV*, muscle volume; *MT*, muscle thickness; *LBM*, lean body mass.

Table 9.2 Details of training protocols in each study.

Study	No. of subjects (%women)	Age (years, mean ± SD)	Training intensity (% 1 RM or nRM)	Duration (frequency)	Training volume [set × rep (rest)]	Exercise
Thomis et al. (2004)	57 (0%)	18–40	60%–85% 1 RM	10 weeks (3 day/ week)	5 sets of 8–14 repetitions (unknown)	Biceps curls
Charbonneau et al. (2008)	243 (57%)	50–85	5 RM	10 weeks (3 day/ week)	5 sets of 5 repetitions (30–180 s)	Knee extension
Pescatello et al. (2006)[a]	631 (58%)	24.2 ± 5.0	65%–90% 1 RM	12 weeks (2 day/ week)	3 sets of 6–12 repetitions (2 min)	Biceps preacher curl, biceps concentration curl, standing biceps curl, overhead triceps extension, triceps kickback
Pereira et al. (2013)	139 (100%)	65.5 ± 8.2	40%–75% 1 RM	12 weeks (3 day/ week)	3 sets of 4–10 repetitions (2–3 min)	Bench press, leg extension, counter movement jump, medicine ball throw
Erskine et al. (2014)	51 (0%)	20.3 ± 3.1	80% 1 RM	9 weeks (2 day/ week)	4 sets of 10 reps (2 min)	Knee extension
Gentil et al. (2011)	141 (0%)	21.9 ± 2.1 (RR) 21.8 ± 2.6 (RX) 22.6 ± 4.3 (XX)	8–12 RM	11 weeks (2 day/ week)	2 sets of 8–12 repetitions (unknown)	Leg press, knee flexion, bench press, pull down, sit ups
Sood et al. (2012)	114 (54%)	50–85	50% (first set)-5 RM	10 weeks (3 day/ week)	4–5 sets of 5–20 repetitions (30, 90, 150, 180 s)	Unilateral knee extension
Hand et al. (2007)	128 (55%)	50–85	50% (first set)-5 RM	10 weeks (3 day/ week)	4–5 sets of 5–20 repetitions (30, 90, 150, 180 s)	Unilateral knee extension

Continued

Table 9.2 Details of training protocols in each study—cont'd

Study	No. of subjects (%women)	Age (years, mean ± SD)	Training intensity (% 1 RM or nRM)	Duration (frequency)	Training volume [set × rep (rest)]	Exercise
Kostek et al. (2005)	67 (52%)	52–83	50% (first set)-5 RM	10 weeks (3 day/week)	4–5 sets of 5–20 repetitions (30, 90, 150, 180 s)	Unilateral knee extension
Hong et al. (2014)	83 (0%)	22.5 ± 1.4	75%–85% 1 RM	8 weeks (3 day/week)	3 sets of 8–12 repetitions (3 min)	Shoulder press, bench press, pull down, arm curl, hammer curl, triceps extension, dips, crunches
Walsh et al. (2009)[a]	754 (60%)	18–39	65%–90% 1 RM	12 weeks (2 day/week)	3 sets of 6–12 repetitions (2 min)	Biceps preacher curl, biceps concentration curl, standing biceps curl, overhead triceps extension, triceps kickback
Li et al. (2014)	94 (0%)	18–22	8–12 RM (1–4 weeks) 4–8 RM (5–8 weeks)	8 weeks (3–4 day/week)	1–4 weeks: 3 set of 8–12 repetitions (1 min) 5–8 weeks: 3 set of 4–8 repetitions (1 min)	Bilateral dumbbell incline curls, isokinetic knee extension
Popadic Gacesa et al. (2012)	29 (0%)	21.5 ± 2.7	10 RM	6 weeks (5 day/week)	5 sets of 10 repetition (1 min)	Triceps extension
Erskine et al. (2012)	51 (0%)	20.3 ± 3.1	80%	9 weeks (2 day/week)	4 sets of 10 reps (2 min)	Knee extension
Harmon et al. (2010)[a]	874 (59%)	18–40	65%–90% 1 RM	12 weeks (2 day/week)	3 sets of 6–12 repetitions (2 min)	Biceps preacher curl, biceps concentration curl, standing biceps curl, overhead triceps extension, triceps kickback

Table 9.2 Details of training protocols in each study—cont'd

Study	No. of subjects (%women)	Age (years, mean ± SD)	Training intensity (% 1 RM or nRM)	Duration (frequency)	Training volume [set × rep (rest)]	Exercise
Riechman et al. (2004)	153 (50%)	18–31	80% 1 RM	10 weeks (3 day/ week)	3 sets of 6–10 repetitions (30 s)	Chest press, seated row, lateral pulldowns, leg extension, triceps extension, arm curl, shoulder press, hamstring curl, low back extension, abdominal crunch and incline press
Pistilli et al. (2008)[a]	748 (60%)	18–40	65%–90% 1 RM	12 weeks (2 day/ week)	3 sets of 6–12 repetitions (2 min)	Biceps preacher curl, biceps concentration curl, standing biceps curl, overhead triceps extension, triceps kickback

RM, repetition maximal.
[a] Functional SNPs Associated with Human Muscle Size and Strength (FAMuSS) cohort.

base pairs in intron 16 on chromosome 17 has shown an association to cardiac risk factors, muscle phenotype, and sports performance (Williams et al., 2000).

Pescatello et al. (2006) examined the association between the ACE I/D polymorphism and the response of the elbow flexors to resistance training. This was one of the first papers generated from the recently completed functional single nucleotide polymorphisms associated with human muscle size and strength (FAMuSS) study, which was designed to identify genetic factors underlying the muscle response to resistance training. The FAMuSS study provides fertile ground for identifying key genes: the sample size is large, measurements of upper-arm muscle size and strength are state of the art, and the training design allows comparisons of trained unilateral and untrained contralateral limbs. In their study, Pescatello et al. (2006) found only minor associations between the ACE genotype and training responses. Specifically, ACE I-allele carriers demonstrated a greater isometric strength increase with resistance training than the D/D group for the trained unilateral arm. However, the 1RM and muscle size responses were not related to the ACE genotype. In contrast, the most interesting results were for the untrained

contralateral limb, where increases in isometric strength, 1 RM, and muscle size were different from the ACE genotypes. Overall, the results indicate that the muscle response to resistance training is not highly related to the ACE I/D polymorphism, whereas the contralateral effects of unilateral training are associated with the presence of the D-allele.

Charbonneau et al. (2008) also examined the effect of the ACE I/D genotype on strength and muscle volume after knee extensor to resistance training. In this study, 86 inactive men and 139 inactive women, ages 50–85 years, performed 10 weeks of unilateral knee extensor resistance training. Their results suggested that the ACE I/D genotype was associated with baseline differences in muscle volume, but it was not associated with the muscle hypertrophic response to resistance training. Thomis et al. (2004) have shown no association between the ACE genotype and response to resistance training after 10 weeks of arm curling exercise. Yet, previous studies present conflicting results regarding the potential role of the ACE genotype effecting a response to resistance training.

9.2.2 ACTN3 R577X genotype alone and in combination with ACE I/D genotype

ACTN3 R577X and ACE I/D genotypes are the most studied gene polymorphisms for athletic performance and muscle phenotypes. ACTN3, actin-binding protein, is the major structural component of the Z-line in skeletal muscles (Beggs et al., 1992). ACTN3 is encoded by ACTN3 on chromosome 11q13.1 (North et al., 1999). ACTN3, normally expressed in fast-twitch skeletal muscle fibers (North et al., 1999), is important for anchoring actin and plays a regulatory role in coordinating muscle fiber contraction (Garton et al., 2014). ACTN3 is absent at a prevalence of approximately 18% in European populations and 25%–29% in the Japanese population. These individuals are homozygous for an allele encoding a premature stop codon at the ACTN3 R577X polymorphism (rs1815739, C-to-T transition at nucleotide position 1729 in the ACTN3 open reading frame) (North et al., 1999). The α-actinin-3 (*ACTN3*) R577X polymorphism is associated with muscle fiber type (Vincent et al., 2007), power (Kikuchi et al., 2014), and elite performance (Gomez-Gallego et al., 2009; Yang et al., 2003) in athletes and in the general population.

Erskine et al. (2014) reported on 51 untrained healthy Caucasian males that performed unilateral knee extension resistance training 3 times a week for 9 weeks on 80% of 1 RM. They found that ACTN3 R-allele carriers demonstrated larger muscle volume, greater power, and strength than XX homozygotes in the untrained state (baseline), but the responses to resistance training were unrelated to the ACTN3 genotype. Furthermore, while the ACE I/D polymorphism was not individually associated with muscle phenotype or training response, when combined with the ACTN3 R577X SNP, the "optimal" genotypes (ACTN3 RR + RX genotype and ACE DD genotype) were associated with greater strength and maximum power. Delmonico et al. (2007) studied an association between the ACTN3 genotype and training responses in a large

Caucasian population. Briefly, 157 relatively healthy sedentary Caucasian men and women participated in their study. The resistance training program was performed on Keiser A-300 air-powered leg extension machines and consisted of unilateral (one-legged) resistance training of the knee extensors of the right leg, 3 times per week, for approximately10 weeks. This study reported that women with XX genotype had greater relative training responses but these differences were not seen in men. Contrary to the results reported by Clarkson et al. (2005), women with RR genotype had greater increases in muscle power, but results in men did not achieve significance. Further, 355 women and 247 men, ages 18–40 years, performed 12 weeks of forearm flexor and extensor resistance training. The exercise progression used the following weekly training protocol: weeks 1–4: 3 sets with 12 repetitions of the 12-repetition maximum weight; weeks 5–9: 3 sets with 8 repetitions of the 8-repetition maximum weight; and weeks 10–12: 3 sets with 6 repetitions of the 6-repetition maximum weight. A study published by Gentil et al. (2011) detailed the outcomes of 141 young men that performed two sets of 8–12 repetitions of five exercises. ACTN3 R577X polymorphism was not associated with baseline muscle strength or the muscle strength response to resistance training. However, RR and RX genotypes, R allele carriers, showed increases in muscle thickness in response to resistance training.

Pereira et al. (2013) reported the influence of ACTN3 polymorphisms and ACE I/D alone and in combination with muscle strength, power, and functional phenotype in older Caucasian women following a 12-week period of high-speed power training. Specifically, 139 healthy older Caucasian women participated in this study (age: 65.5 ± 8.2 years, body mass: 67.0 ± 10.0 kg, and height: 1.57 ± 0.06 m). The training consisted of progressive weight loads with 3 sets of 10 reps with a load of 40% of 1 RM at the outset of their predetermined 1-repetition maximum, increasing to 3 sets of 4 reps with a load of 75% toward the end of the 12-week period in 1 RM. They suggested that both the ACE I/D and ACTN3 R577X polymorphisms (alone or in combination) were strong potential factors in modulating some exercise-related phenotypes induced by training-induced responses that affect muscle adaptation to resistance training. In the study by Lima et al. (2011), 246 women (age 66.7 ± 5.5 years) participated in the study. The program followed a progressive intensity, with training loads of 60% of 1 RM in the first 4 weeks, 70% in the following 4 weeks, and 80% in the remaining 16 weeks, with repetitions, respectively, decreased from 12, 10, and 8. Each exercise was performed in 3 sets with an approximately 1-min rest between sets. The findings did not support a pivotal role of the ACE I/D and ACTN3 R577X polymorphisms in determining muscle strength, either at baseline or in adaptation to a RT program, in older women.

The R577X polymorphism at the *ACTN3* gene has been associated with muscle strength, hypertrophy, and athletic status. The X allele, which is associated with the absence of ACTN3 protein, is suggested to impair performance of high force/velocity muscle contractions. In all, 141 men performed two resistance training sessions per week for 11 weeks. Participants were tested for 1RM bench press, knee extensors peak torque,

and knee extensors muscle thickness at baseline and after the training period. Genotype distribution was 34.4% for RR, 47% for RX, and 18.6% for the XX genotype. According to the results, the R577X polymorphism at *ACTN3* was not associated with baseline muscle strength or the muscle strength response to resistance training. However, only carriers of the R allele showed increases in muscle thickness in response to training (Gentil et al., 2011).

9.2.3 IGF-1 genotype

The contribution of insulin-like growth factor I (IGF-I) to muscle mass and function across the entire life span has been well established (Borst and Lowenthal, 1997). Low-plasma IGF-I levels are associated with slow walk speed and self-reported difficulty in mobility tasks, suggesting a role of IGF-I in disability and frailty in older populations (Cappola et al., 2001). Previous studies have shown that resistance training increases IGF-I mRNA and protein levels in skeletal muscle (Psilander et al., 2003; Hameed et al., 2004). Moreover, IGF-I expression is associated with muscle hypertrophy (Philippou et al., 2007), specific force and intracellular calcium in a muscle, and motor neuron regeneration and preservation of fast muscle fibers in aged muscle.

The common IGF-1 $(CA)_{19}$ microsatellite allele may alter transcription through its effect on regulatory elements (Mccarthy et al., 1997) and has been associated with decreased circulating levels of the IGF-1 protein in some studies (Rietveld et al., 2003) but not all. This polymorphism is also associated with functional properties and phenotype linked to the IGF-1 protein (Allen et al., 2002). Some studies demonstrate an association between an IGF-I CA repeat polymorphism and baseline strength and muscle volume and training response to resistance training. Kostek et al. (2005) and Hand et al. (2007) reported no significant differences in baseline muscle strength or muscle volume among IGF-I CA repeat genotype groups. On the other hand, Sood et al. (2012) suggested that the noncarriers of the 192 allele had the highest peak power values, followed by the 192 heterozygotes and 192 homozygotes, respectively, especially in women. Kostek et al. (2005) found a significant influence of the IGF1 genotype on muscle strength response to resistance training. In addition, Hand et al. (2007) reported a significant IGF-I main effect, as well as a significant genotype gene-by-gene interaction effect of IGF-I and calcineurin B (PPP3R1) for change in strength with resistance training. Those results indicated that the IGF-I genotype is associated with metabolic stress, such as IGF-1 responses, and this is necessary to achieve muscle hypertrophy during resistance training.

9.2.4 Interleukin-15 and interleukin-15 receptor genotype

Previous studies have identified IL-15 as an anabolic cytokine in myogenic cultures. Myotubes of murine (Quinn et al., 1995), bovine (Quinn et al., 1995), and human

(Furmanczyk and Quinn, 2003) origin all increase the protein content of heavy chain myosin when IL-15 is included in culture media. IL-15 mRNA increased in the muscles of aged animals and in muscles exposed to atrophic stimuli, such as hind limb suspension and unweighting, following stretch-induced hypertrophy (Ford, 2005). Collectively these data demonstrate that IL-15 expression within skeletal muscle is affected by conditions that promote muscle atrophy and that IL-15 may be able to counter muscle loss induced by disease. There are two studies that performed the association between IL-15 and IL-15 receptor gene variants and response to resistance training. In the study by Pistilli et al. (2008), it was reported that the association between two IL-15 polymorphisms (rs1057972, rs1589241) and three IL-15RA polymorphisms (rs3136618, rs2228059, 2296135) were responsive to progressive 12-week unilateral upper-arm resistance training. Specifically, The C-allele in the 3′ untranslated region of the rs2296135 IL-15Rα polymorphism was associated with greater improvements in posttraining isometric strength, only in females, in large cohort of FAMuSS ($n = 748$). The study by Riechman et al. (2004) used 153 male and female Caucasian subjects who performed a 10-week total body progressive resistance training program. Three variants of the IL-15Rα polymorphism (rs3136617, rs3136618, and rs2296135) were genotyped. The participants performed 3 days a week at 75% 1 RM for 10 weeks, undertaking 3 sets of 13 resistance exercises. Muscle adaptations were determined using circumference measures of the upper arm and upper leg musculature to assess changes in muscle size while 1RM was used to determine changes in muscle strength. They suggested that the exon 7 PstI C to A polymorphism in the *IL-15 RA* gene was associated with greater muscle mass gains but reduced muscle quality gains. Moreover, *IL15RA* exon 4 (BstNI) genotypes or BstNI/HpaII haplotypes were also significantly associated with lean mass gain independent of the PstI variation. Interestingly, the conflicted trend was observed in rs2296135 polymorphism and *IL-15 RA* as a change of muscle strength and muscle volume with resistance training, respectively. Further investigation is needed to explore genetic variants in the *IL-15* and *IL-15RA* genes for their association with physiological response to resistance training.

9.2.5 CCL2 and CCR genotype

Chemokines (chemotactic cytokines) are small proteins that direct circulating leukocytes to an area of inflammation and injury (Charo and Ransohoff, 2006). One of the most thoroughly characterized members of this family is monocyte chemoattractant protein 1, also known as chemokine ligand 2 (CCL2). CCl2 and one of its major receptors, monocyte chemoattractant protein 1 receptor (CCR2), are coded by the *CCL2* and *CCR2* genes, respectively. Initial interest in CCL2 as a potential modifier of muscle arose from studies that showed a significant upregulation of CCL2 and CCR2 after muscle injury and during muscle regeneration.

Harmon et al. (2010) reported that the association between *CCL2* and *CCR2* gene variants and muscle strength and change in strength with resistance training in the FAMUSS cohort. They genotyped 11 genetic variants in the chemokine gene *CCL2* (rs17652343, rs1860189, rs3917878, rs2857654, rs1024611, rs1024610, rs3760396, rs2857656, rs2857657, rs4586, and rs13900) and five variants in the chemokine receptor gene CCR (rs17141010, rs768539, rs3918358, rs1799864, and rs1799865). Subjects ($n = 874$) underwent a 12-week resistance training program for the upper arm muscles. Muscle size and elbow flection strength measurements were taken before and after training. Results showed four variants in *CCL2* (rs1024611, rs2857656, rs4586, and rs13900) were associated with baseline strength and one variant (rs1024610) was associated with an increase in strength in response to the 12 weeks of resistance training. In addition, they found one variant (rs768539) in *CCR* was associated with baseline strength and two variants (rs3918358 and rs1799865) were associated with an increase in strength. These associated SNPs explained 0.7%–2.5% of the phenotype.

9.2.6 MSTN genotype

Myostatin, encoded by *MSTN* at the human chromosomal locus 2q32.2, is a secreted growth factor critical for the regulation of skeletal muscle myogenesis (Mcpherron and Lee, 1997). Evidence of a role of myostatin in skeletal muscle growth and development first came from observations that *MSTN*-null animals exhibited hypermuscularity or "double-muscling." In particular, mice carrying a targeted inactivation of the gene were shown to exhibit a 200%–300% increase in skeletal muscle mass (Mcpherron and Lee, 1997), and several other groups also showed that mutations in the gene can lead to a visibly distinct muscular hypertrophy in cattle (Grobet et al., 1997; Mcpherron et al., 1997). More recently, Schuelke et al. (2004) reported the presence of a loss-of-function mutation in *MSTN* in a child with an extraordinarily muscular phenotype. Additionally, transgenic mice with muscle-specific overexpression of myostatin protein had lower skeletal muscle mass (Reisz-Porszasz et al., 2003), which further indicates an essential role of the secreted protein as a negative regulator of muscle growth.

Li et al. (2014) reported that A55T and K153R polymorphisms of *MSTN* were associated with strength training-induced muscle hypertrophy. The participants performed 3 sets of 8–12 RM for each exercise during the first 4 weeks of the training program and 3 sets of 4–8 RM during the subsequent 4 weeks, with a 60-s resting period allowed between each set. This study demonstrated that the variant alleles of *MSTN* A55T and K153R polymorphisms could significantly enhance muscle hypertrophy in response to strength training among Han Chinese men. Even before the participants underwent the strength training program, the thicknesses of biceps and quadriceps were greater among individuals with the variant alleles than those with wild-type alleles. These observations suggest that the polymorphisms could not only result in larger muscle size under

untrained conditions, but the increase in muscle size following strength training would also be more prominent if an individual carried the variant alleles of the polymorphisms.

9.2.7 B2BRK genotype

Bradykinin is a nonapeptide that acts as a potent vasodilator. Within skeletal muscle, bradykinin promotes glucose uptake and alters muscle blood flow (Wicklmayr et al., 1983). Bradykinin exerts many of its effects by binding to bradykinin type 2 receptor (B2BRK). Several studies have implicated B2BRK in exercise adaptation and regulation of skeletal muscle performance. Response to a 10-week training program resulted in changes in the left ventricular mass, which were greatest for carriers of the +9 allele and smallest for the −9 homozygotes (Brull et al., 2001). The bradykinin −9 allele was also associated with greater skeletal muscle metabolic efficiency in both athletes and nonathletes (Williams et al., 2004). However, there is only one report on the effect of B2BRK 9-bp polymorphism on the skeletal muscle response to strength training (Popadic Gacesa et al., 2012). Popadic Gacesa et al. (2012) examined the influence of the B2BRK 9-bp polymorphism on triceps brachii muscle hypertrophy as a result of a 6-week self-perceived maximal elbow extensors strength training. In this study, 29 healthy young Caucasian men (21.5 ± 2.7 years), who did not take part in any formal resistance exercise regime for 6 months prior to starting a 6-week exercise protocol, volunteered. Elbow extensors of −9/−9 B2BRK homozygous individuals exhibited significantly higher hypertrophy compared to those with −9/+9 or +9/+9 B2BRK variants. However, no significant influence of different B2BRK genotypes on functional muscle properties after strength training in young healthy untrained subjects was found. There are some limitations. Especially, the number of participants was small in −9/−9 and +9/+9 genotype groups (only 6 and 5, respectively). Future studies are necessary to determine the effects of B2BRK genotypes on the response to resistance training.

9.2.8 PTK2 genotype

The focal-adhesion tyrosine kinase, FAK, has been shown to play a major role in costamere formation and turnover (Cary and Guan, 1999; Quach and Rando, 2006), and FAK expression is controlled at the level of the protein tyrosine kinase-2 (*PTK2*) gene. Therefore, polymorphisms of *PTK2* could potentially underpin the considerable interindividual variability reported in untrained human muscle specific force (range 22–40 N/cm^2 (Erskine et al., 2009)) and in the training-induced relative change in specific force, which varies between 5% and 39% (Erskine et al., 2010). Erskine et al. (2012) investigated whether associations existed between PTK2 polymorphisms and human skeletal muscle strength phenotypes before and after resistance training (9 weeks of high-intensity unilateral knee extension resistance training in 51 previously untrained men). In addition, all participants had blood samples isolated, which were genotyped

for the *PTK2* rs7460 A/T and rs7843014 A/C SNPs. The two *PTK2* polymorphisms were significantly associated with interindividual variability in muscle specific force; however, it did not contribute to the observed interindividual variation in the training response.

9.2.9 CNTF genotype

Ciliary neurotrophic factor (CNTF) facilitates motor nerve function (Sendtner et al., 1992). CNTF is also a cytokine belonging to the interleukin (IL)-6 family, and when combined with the CNTF receptor, it performs the role of a chemical messenger for target tissues, such as motor nerves and skeletal muscles (Ip et al., 1993). Since Takahashi et al. (1994) first reported the substitution of the human *CNTF* gene 1357 G→A SNP, several studies have investigated the relationship between *CNTF* polymorphisms and muscle function. Large-scale, cross-sectional studies have demonstrated that individuals possessing *CNTF* variants showed greater muscle strength than those with the G1357G genotype (Roth et al., 2001; De Mars et al., 2007). Roth et al. (2001) examined the role of this *CNTF* genetic variant as it relates to muscle strength in 494 men and women aged 20–90 years. Subjects heterozygous for the null mutation of *CNTF* 1357 G → A SNP exhibited significantly greater concentric peak torque (180 degrees/s angular velocity) in knee extensors and flexors than individuals with the G1357G genotype. Using Biodex dynamometry, De Mars et al. (2007) investigated genetic variations in *CNTF* and *CNTFR* and their relationships to muscle strength of the knee extensors and flexors in 493 men and women aged 38–80 years. They found an association between *CNTF* 1357 G → A polymorphism and strength in men and showed inconclusive results for a limited number of strength phenotypes in women. However, the effects of resistance training varied depending on the *CNTF* polymorphism, that is, women with the GG genotype showed a greater increase in muscle strength after 12 weeks of training than those with the AA genotype, whereas men showed no difference in strength based on genotype (Walsh et al., 2009). Hong et al. (2014) suggested that 8 weeks of resistance training resulted in improvements in motor unit potential and muscle strength and endurance, and no differences were associated with the *CNTF* genotype, except for the biceps brachii muscle during 180 degrees/s exercises. Therefore, improvements in muscle strength and endurance after resistance training in healthy male college students were a direct result of the training program and were not related to a *CNTF* genotype.

9.3 Scoring genetic polymorphisms for full utility in training

In 2016, an article was published in the *Biology of Sports* journal entitled "A genetic-based algorithm for personalized resistance training," which suggested that training efficiency could be improved by utilizing genetic information for training protocols (Jones et al., 2016). In the report by Jones et al. (2016), each scored 0–4 points (the

14 genes and 15 polymorphisms), and the individual differences in training response were investigated by classifying the subjects into endurance type (E) or power type (P) based on the scores. Two types of evaluation measurements, counter movement jump and an aerobic 3-min cycle test (Aero3), were taken before and after 8 weeks of training, either low-intensity resistance training or high-intensity resistance training, in a total of 67 subjects, comprised of 28 male athletes (Study 1) and 39 male soccer players (Study 2). In low-intensity training, the athletes performed a high number of repetitions (10–20 repetitions), while in high-intensity training, the athletes performed a low number of repetitions (2 repetitions). The results of the study showed that athletes with endurance-type genetic scores who performed low-intensity training tended to have a higher rate of improvement in counter movement than athletes with power-type genetic scores (E: 7.6% vs P: 2.3). Conversely, athletes with power-type genetic scores in the group that performed high-intensity training tended to have a higher rate of improvement in counter movement jump than athletes with endurance-type genetic scores (E: 2.8% vs P: 7.1). A similar tendency was demonstrated with the improvement rate of Aero3. In other words, in both measurement items, the counter movement jump, which is an evaluation of explosive muscle power, and Aero3, which is an evaluation of endurance capacity, athletes with endurance-type genetic scores had a high training effect with low-intensity training and athletes with power-type genetic scores had a high training effect with high-intensity training. Although the report by Jones et al. has room for further investigation, the results suggest that genetic types may be used as evaluation criteria for determining training intensity.

In 2016, two contrasting Letters to the Editor were published in the *Biology of Sports* journal in response to the article by Jones et al. (Karanikolou et al., 2017; Monnerat-Cahli et al., 2017). Monnerat-Cahli et al. entitled their letter "Are the doors opened to a genetic-based algorithm for personalized resistance training?" and claimed that the article by Jones et al. was a breakthrough, and they believed that there would be many similar articles published in the future (Monnerat-Cahli et al., 2017). Several studies, like the study by Jones et al., on the training effect based on scoring of genetic polymorphisms and genetic polymorphism profiles would be needed to apply genetic polymorphisms to training instruction.

Conversely, Karanikolou et al. (2017) countered that the article by Jones et al. was premature. Karanikolou et al. (2017) claimed that only 5 of the 15 polymorphisms reached a level of significance after Bonferroni correction ($p < 0.003 = 0.05/15$) in the article by Jones et al. in terms of the relevance of the genetic polymorphisms used to the counter movement jump or the Aero3 training effect. Furthermore, as shown in Table 9.2, they pointed out that the study included polymorphisms with few reports, and there were no reports on the direct relevance of endurance performance to CRP genetic polymorphisms. They also raised the issue that the extremely small sample size in the study was problematic in the field of genetic research. If we look at genetics

research on training effects, in the studies by Bouchard et al. and Timmons et al. described in the beginning of this chapter, both studies claimed the ability to predict a maximum oxygen uptake of 23%–49% using a number of genetic polymorphisms; however, the polymorphisms said to be related to genes in the two studies did not match. Even with the *ACTN3* R577X polymorphisms, which have been the focus of much research, particularly in the field of sports performance and genetic polymorphisms, the contribution rate to muscle strength in general subjects is low, at 2%–3%. The results of conducting the Wingate test on 256 university athletes in our study indicated that the contribution rate of *ACTN3* R577X polymorphisms to peak power per kg of weight was 4.6% (Kikuchi et al., 2014). When scoring a number of genetic polymorphisms, the rate of contribution on the training effect of each polymorphism is expected to differ; therefore, determining how to score specific genetic polymorphisms is an issue to be investigated in the future.

To summarize the claims by Karanikolou et al., there are three points as follows: (1) there is insufficient scientific basis for the related genetic polymorphisms, (2) the algorithm is unclear, and (3) the sample size is too small. Jones et al. put forward a further counterargument to the claims by Karanikolou et al. (Jones et al., 2017). The major focus of these comments was that the 2016 article (Jones et al., 2016) was "a controlled intervention study and not genetic research." Genetic research requires extremely large sample sizes due to the tendency for type 1 errors (Sarzynski et al., 2016). Discussion is needed on the implementation method of interventional studies that contain genetic data. Further, the description of the training method and evaluation methods used in the study by Jones et al. need more consideration, so future interventional studies that handle genetic data in this way must proceed with the collaboration of the coach, supervisor, training instructor, and researcher.

9.4 Conclusion

When creating a training program, the current situation must first be ascertained by conducting a needs analysis, measurement, and evaluation. Subsequently, events should be set to suit the subjects and the aims of the study, setting variables such as intensity, volume, and frequency. While it is important to conduct training in line with the aim of the study, the recent development of sports science and equipment enables multifaceted approaches to achieve a single goal. Therefore, it is essential to provide instruction based on consideration of the subjects' characteristics. It is often impossible to evaluate these individual characteristics with measurements of physical strength elements alone. Furthermore, with future clarification of genetic polymorphisms related to risks, such as injury and fatigue resistance during training, instructors may be able to change their approach during instruction based on knowledge of an athlete's genetic characteristics when providing training instruction.

Genetic information is the ultimate personal information. Therefore, extreme caution is needed when using this information, and it is important to understand this information is a tool for demonstrating the characteristics of an individual, similar to measured data on physical strength. As a result, this information will serve as an aid for searching for methods to make the most of individual characteristics and may provide methods that will lead to further improved performance in training practice. Furthermore, the reality is that utilizing genetic information in the field of sports coaching poses ethical and social issues, as mentioned at the beginning of this report, and there are still many coaches and athletes who are resistant to these methods. It is vital to gather more evidence through genetic research in sports and establish this information as a tool that can be utilized in practice through trial and error. We hope that achieving this end will facilitate training instructions utilizing genetic information, including determining the content, volume, and intensity of the training depending on the combination of genetic polymorphisms in any one individual and predicting the training effect.

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CHAPTER TEN

Exercise and DNA methylation in skeletal muscle

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10.1 Introduction

Exercise is an external stressor that leads to an extensive coordinated response in several organs and tissues. If exercise is chronically repeated this leads to adaptation of those organs and tissues at both the macroscopic (organ and tissue) and microscopic (cellular and molecular) level. Exercise physiologists have begun to elucidate the cellular and molecular responses to exercise that ultimately lead to adaptation at the whole tissue and systems level. Exercise, depending on the mode (e.g., aerobic or resistance), leads to the production of numerous extracellular molecules produced by various glands and tissues such as (and not limited to); calcium, hormones (testosterone, cortisol, growth hormone), growth factors (insulin, insulin-like growth factors), catecholamines (epinephrine, norepinephrine), cytokines (TNF-alpha, IL-6), and nitric oxide. These extracellular molecules/ligands then bind to specific cell surface receptors (if protein-based molecules) or pass through cell membranes (if lipid based) in the target tissue. In the case of nonprotein molecules such as calcium or potassium, they are transported via transmembrane proteins such as ions channels, or via carrier/transport proteins (e.g., glucose is transported via glucose transporter proteins, such as GLUT1). Once bound to membrane specific receptors, or translocated across the cell surface, these extracellular molecules bring about a cascade of intracellular signaling responses. These responses usually change the activity of specific proteins, which in-turn, leads to an activation/deactivation of downstream transcription factors. There are some exceptions to this, an example would be the lipid-based molecule testosterone, that has an intracellular receptor called androgen receptor (AR), that once bound to its ligand, translocates from the cytosol to the cell nucleus and acts as its own transcription factor, without the requirement for changing intracellular protein activity/signaling. Transcription factors then bind to DNA to promote or silence (turn on or off) gene expression (mRNA transcription).

Sports, Exercise, and Nutritional Genomics
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Ultimately, in most cases (but not all due to posttranscriptional modifications of mRNA), the transcriptional level of mRNA for a particular gene then determines the amount of translated peptides that are synthesized by the ribosome. The mature peptide or peptides (proteins) are then able to perform their function in maintaining cellular homeostasis, and upon chronic, repetitive exposure to certain exercise stressors, increases in protein abundance over time can lead to significant adaptations at both the cellular and tissue level (Perry et al., 2010).

Epigenetics is an emerging area within molecular exercise physiology, referring to changes in gene function, such as mRNA expression or RNA sequence, that are not due to changes in the DNA sequence itself. The changes are brought about by several modifications to DNA or the surrounding histones following exposure to environmental stimuli that modify the conformational characteristic of the macromolecule, helping to promote or suppress the process of transcription, and therefore the subsequent level of gene expression. DNA methylation is the most common modification to DNA itself, where there is an addition of a covalent methyl group to position 5 of the pyrimidine ring of a cytosine (5mC) (Fig. 10.1). The majority of annotated gene promoters contain cytosine-guanine (C-G) base pairing (CpG dinucleotide/site) rich regions, known as CpG islands. In humans, the majority of CpG sites (70–80%) are methylated (Lister et al., 2009; Ziller et al., 2013), and CpG methylation in promoter or enhancer regions usually leads to an inability of the transcriptional apparatus to bind to gene regulatory portions of a gene (Bogdanovic and Veenstra, 2009). Furthermore, via the recruitment of chromatin modifying protein/protein-complexes, methylated CpG islands in promoters create a tight compaction to adjacent chromatin (termed heterochromatin) that prevents transcription initiation. Therefore, increased (hyper) methylation of CpG sites or multiple sites in a CpG island, especially in enhancer or promoter regions, generally silences gene expression. Whereas, decreased (hypo) methylation generally enhances gene expression. DNA methylation is also regulated by DNA methyltransferases (DNMTs) (Fig. 10.1). DNMT1 helps maintain methylation once the modification has occurred, whereas DNMT 3a and b control de novo methylation of a gene (Trasler et al., 2003). Alternatively, DNA methylation removal (demethylation) is performed via two methods; (1) active demethylation, occurs via the ten-eleven translocation (TET) enzymes, including TET1, 2 and 3 that convert the 5mC to 5-hydroxymethyl cytosine (5hmC) which then removes the methyl group through a base excision repair mechanism (Tahiliani et al., 2009; Ito et al., 2010) (Fig. 10.1). (2) Passive demethylation, occurs through a TET independent manner, where DNA methylation is not maintained via DNMT1 during replication and is therefore passively lost (Fig. 10.1).

Another important epigenetic modification that affects DNA is that of the surrounding core proteins of chromatin complexes, called histones, including; histone(H) 2A, H2B, H3, and H4. These histones provide a core structure for DNA to be wound at regular intervals into chromatin (Luger et al., 1997). Histones are prone to posttranslational changes such as methylation, acetylation, phosphorylation, ubiquitination,

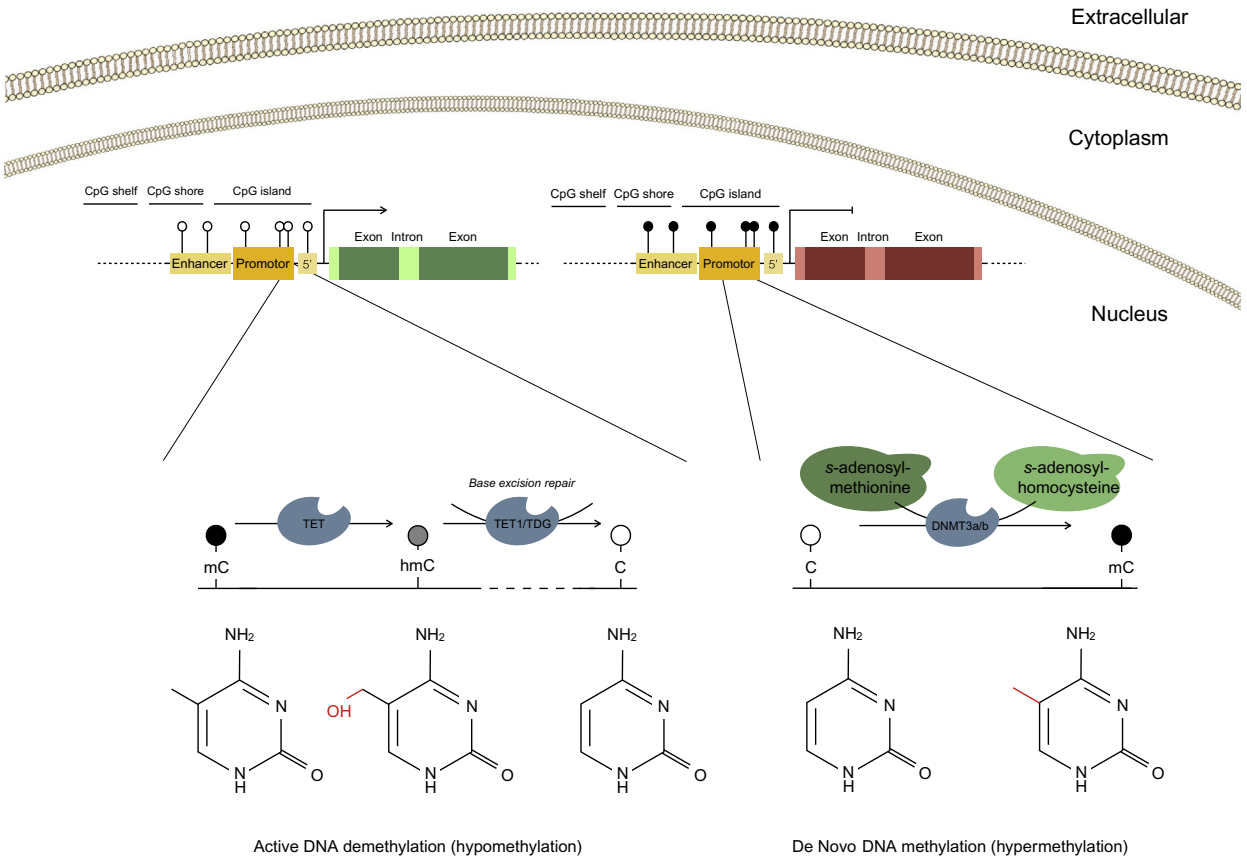

Fig. 10.1 The process of DNA methylation (hypermethylation) and demethylation (hypomethylation) and its enzymatic regulation. *C*, cytosine; *CpG*, cytosine-guanine base pairing; *DNMT*, DNA methyltransferase; *hmC*, hydroxymethylcytosine; *mC*, methylcytosine; *TDG*, thymine DNA glycosylase; *TET*, ten-eleven translocation enzyme.

SUMOylation, and citrullination due to their long N or C terminal residues that protrude out from the globular structure of the histone octamer (Bannister and Kouzarides, 2011). Histone modifications lead to the DNA being rendered into either a more compact (repressive/inhibitory/heterochromatin) or relaxed (permissive/allowing/euchromatin) state that subsequently alters access for the transcriptional machinery regulating gene expression. The process of specific histone modifications leading to alterations in gene expression is more varied than direct DNA CpG site/island methylation, depending on the scenario. However, there are certain modifications that tend to alter gene expression in a similar way when they occur in the majority of cases. This includes trimethylation of H3 on lysine (K) 4 (H3K4me3) and acetylation of various lysine residues of H3 and H4, which are associated with increased gene expression and are therefore termed permissive histone modifications. Conversely, trimethylation of H3 lysines in the 9th and 27th position (H3K9me3 and H3K27me3), as well as lysine 20 trimethylation on H4 (H4K20me3) leads to a repressive effect on gene expression (Schuettengruber et al., 2011). Furthermore, acetylation (addition of an acetyl group) to histones are generally associated with increased gene expression as these modifications can help relax chromatin structure and enable transcription, whereas deacetylation (addition of an acetyl group) results in more compact chromatin and is therefore associated with reduced gene expression (reviewed in Wang et al., 2009). The histone acetyltransferases (HATs) and deacetylases (HDACs) control acetylation and deacetylation, respectively. The HATs include: P300/CBP-associated factor (PCAF), K lysine acetyltransferase 2A (GCN5), CREB-binding protein (CBP), p300, K lysine acetyltransferase 5 (Tip60), and Male absent on the first (MOF). These HATs are involved in the addition of acetyl groups to target histones. HDACs include class I (HDAC 1-3 and 8), class II (HDAC 4-7, 9-10), class III (Sirtuins 1-7), and class IV HDACs (HDAC11) (Bannister and Kouzarides, 2011; Wang et al., 2009). The final major epigenetic modification is via micro-RNAs (miRNAs or miRs) that can also affect gene function. These are noncoding RNA sequences, typically 20–30 nucleotides in length, that can modify genes posttranscriptionally. This mainly occurs via impairing gene translation and destabilizing the mRNA molecule (Carthew and Sontheimer, 2009). While not always the case, the effect of miRs is therefore typically inhibitory to gene function. Despite miRs only making up 1% of the genome, approximately 30% of genes have been hypothesized to be miR targets (Lewis et al., 2005), demonstrating they have a wide variety of important epigenetic functions.

10.2 Aims and scope

The area of DNA methylation and exercise has recently seen a rapid expansion in the number of original research articles in a short-time frame, with the first studies only emerging approximately 5–6 years ago. Furthermore, there have been recent extensive reviews on the role of miRs (Wang et al., 2017), and relatively few empirical studies with

respect to histone modifications and exercise adaptation. With this in mind, this chapter aims to review the role of DNA methylation in response to exercise. Furthermore, as skeletal muscle is the predominant tissue that is used for experimentation to investigate the mechanisms of exercise adaptation, we aim to provide an overview of the studies that have examined DNA methylation in response to acute and chronic aerobic and resistance exercise, specifically in skeletal muscle. Finally, we discuss and highlight the most current research into a relatively new phenomenon called epigenetic muscle memory. Where there has been recent evidence emerging to suggest that the methylation status of DNA can be retained after acute or chronic resistance exercise, even during periods of exercise cessation or detraining, that subsequently may lead the epigenome to be in an advantageous position for altering gene expression and therefore physiological adaptation when later retraining occurs.

10.3 Aerobic exercise and DNA methylation in skeletal muscle

PGC-1α has been long established as an important regulator of mitochondrial biogenesis (Puigserver et al., 1998; Wu et al., 1999). It was first demonstrated to be increased in skeletal muscle after aerobic exercise in rodents (Baar et al., 2002) and later in humans (Pilegaard et al., 2003), with exercise performance impaired after its gene knock-out in an animal model (Handschin et al., 2007). Early work into the role of DNA methylation in skeletal muscle identified that PGC-1α DNA methylation was inversely associated with PGC-1α gene expression and mitochondrial DNA (mtDNA) levels (Barres et al., 2009). With these studies also demonstrating that increased PGC-1α methylation was associated with reduced PGC-1α gene expression in skeletal muscle of type-II diabetics vs normal glucose tolerant individuals, who had relatively reduced PGC-1α methylation and increased gene expression in comparison (Barres et al., 2009). Following this work, a study suggested that there was a trend toward hypermethylation of the PGC-1α promoter and corresponding decrease in its transcript expression in human skeletal muscle after a sudden reduction in physical activity (bed rest) (Alibegovic et al., 2010). In 2012, the first direct exercise studies in humans investigated DNA methylation of PGC-1α, and demonstrated a reduction postacute aerobic exercise (at 80% VO_{2max} until 1674 kJ/400 kcal was expended) together with the same trend observed for other mitochondrial transcripts including, mitochondrial transcription factor A (TFAM) and pyruvate dehydrogenase lipoamide kinase isozyme 4 (PDK4) (Barres et al., 2012). Three hours post the same exercise there was also a reduction in PGC-1α, PDK4 as well as peroxisome proliferator-activated receptor (PPAR-δ) methylation, corresponding with an increase in the expression of the same genes (Barres et al., 2012). Interestingly, the work also suggested that higher exercise intensities were required to elicit these changes in DNA methylation. Where aerobic exercise, matched for energy expenditure at 40% VO_{2max}, resulted in no changes in methylation of the same genes vs the higher intensity of 80% VO_{2max} (Barres et al., 2012). It is worth noting, that in separate studies, medium-intensity exercise

(120 min steady state VO_{2peak}), despite not reporting PGC-1α methylation, evoked an increase in promoter methylation of metabolic-related genes, including; fatty-acid-binding protein 3 (FABP3) and cytochrome c oxidase subunit 4I1 (*COX4I1*) that resulted in decreased gene expression after 4-h postexercise (Lane et al., 2015). These studies combined therefore suggest that DNA methylation changes occur at both medium and high intensities of exercise. Taking these findings further, it has been suggested that promoter DNA methylation occurs on a particular transcript variant of PGC-1α. Whereas reduced methylation of the canonical promoter (promoter A) was reduced with elevated gene expression after 1 h of aerobic exercise in mice (35 rpm for 20 min, 40 rpm for 30 min, and 45 rpm for 10 min). However, the alternative promoter (B) did not demonstrate reduced DNA methylation, rather this promotor displayed an alteration at the histone level via trimethylation of H3 on lysine 4 (H3K4me3) (Lochmann et al., 2015). Trimethylation of this particular histone has been shown to render DNA into euchromatin (transcriptionally permissive) state and is therefore associated with increased gene expression (Schuettengruber et al., 2011). Despite accumulating evidence that DNA methylation plays a role in PGC-1α expression (and related metabolic genes), there have been limited insights for the role of acute aerobic exercise in modulating other metabolic pathways. Indeed, to the author's knowledge there are currently no studies investigating genome wide DNA methylation after acute aerobic exercise in skeletal muscle. This is therefore an area that warrants further investigation.

It is worth noting genome-wide DNA methylation analysis (29.5 K CpG array that covers promoters of known genes) has been performed after chronic aerobic exercise. Where, following 6 months of supervised aerobic exercise in humans (3 days/week—intensity not defined, albeit VO_{2max} increased after 6 months training), 2051 and 766 genes demonstrated reduced and increased methylation, respectively (P enrichment ≤ 0.005) across all individuals with and without a family history of type-II diabetes (Nitert et al., 2012). In the same study, pathway analysis identified that insulin-signaling, starch and sucrose metabolism, calcium signaling, and retinol metabolism were significantly enriched pathways demonstrating predominantly hypomethylation. Whereas pathways with significantly enriched hypermethylation included purine, glycine, serine, and threonine metabolism as well as glycolysis/gluconeogenesis pathways (Nitert et al., 2012). Therefore, in this study the majority (2051) of genes were hypomethylated (vs 766 hypermethylated), of which included known metabolic genes, myocyte-specific enhancer factor 2A (MEF2A), runt-related transcription factor 1 (RUNX1), NADH dehydrogenase [ubiquinone] 1 subunit C2 (NDUFC2), and thyroid adenoma associated (THADA). Furthermore, using in vitro assays, the authors demonstrated that methylation of these human promoter regions suppressed reporter gene expression in HEK293 cells (Nitert et al., 2012), further suggesting a role for promotor methylation in regulating these gene expression levels. MEF2A and RUNX1 are both genes that code for transcription factors that influence exercise-induced

glucose uptake in skeletal muscle (Smith et al., 2007, 2008). NDUFC2 is a respiratory chain enzyme in mitochondria of muscle (Olsson et al., 2011) and THADA polymorphisms are associated with type-II diabetes (Parikh et al., 2009), suggesting that aerobic exercise-induced hypomethylation of genes that are important in regulating gene expression in these metabolic pathways. Genome-wide analysis (450 K CpG arrays) has also been conducted in obese type-II diabetics after a progressive 16-week endurance exercise program (3 days/week steady-state exercise for 40–60 min at 65%–85% of heart rate reserve) (Rowlands et al., 2014). As in studies described above, these experiments demonstrated that across the genome there was a larger number of CpGs (386 CpG sites) that were hypomethylated vs hypermethylated (169 CpG Sites), and pathway analysis suggested these genes were associated with lipid metabolism, carbohydrate metabolism, metabolic disease, cell death and survival, cardiovascular system development/function, and hematological system development/function (Rowlands et al., 2014). Other studies have further investigated genome wide methylation (450 K arrays) in type-II diabetics in response to chronic aerobic exercise (4 days/week, 10 weeks of progressive intensity) (Stephens et al., 2018). Importantly, this study extended the work above, where the authors characterized their patients into either nonresponders or responders to exercise (assessed by PCr recovery rate after 10 weeks of aerobic exercise). Indeed, the responders and nonresponders demonstrated differential methylation post chronic aerobic exercise across 533 CpG sites. In these identified CpG sites, nonresponders possessed reduced promoter methylation for genes associated with glutathione metabolism, insulin signaling, and mitochondrial metabolism pathways. The most prominent alterations in DNA methylation and corresponding gene expression (analyzed via RNAseq) were found within the glutathione metabolism pathway, particularly on chromosome 1. On this chromosome, both genes GSTM1 and GSTM5 demonstrated hypomethylation across a combined total of 12 CpG sites with associated increases in the same transcripts gene expression (Stephens et al., 2018). Given the studies described earlier suggesting that exercise promoted a genome-wide hypomethylated profile, it may have been hypothesized that responders to exercise would have possessed predominantly a hypomethylated genome with increased gene expression vs nonresponders. However, given the study suggested that nonresponders demonstrated this response, it may be hypothesized that exercise responders start with greater hypomethylation across the genome at baseline, contributing to these results and requiring further confirmative investigation.

Finally, despite these extensive studies on genome-wide epigenetics after chronic aerobic exercise, it is important to note that these studies were conducted in patients with obesity and type-II diabetes. The current authors are only aware of one study that has been conducted in healthy individuals, where 450 K CpG sites were investigated in both young and elderly adults following 12 weeks of chronic aerobic exercise, that consisted of 2 days/week high-intensity interval exercise (>90% VO_{2Peak} 4 × 4 min intervals, 3 min

rest between intervals) and 2 days/week 45-min continuous aerobic exercise (70% VO_{2Peak}) (Robinson et al., 2017). The authors demonstrated that there was less than 10% change in promoter CpG methylation after chronic aerobic exercise. There were however over 3874 promoter CpG sites significantly differentially methylated between young and elderly participants after exercise (Robinson et al., 2017). Of the studies identified within this section, it is important to note that given advances in technology since they were published there are now assays that provide a more comprehensive coverage (850 K array or whole-genome bisulfite sequencing), in which important loci, such as transcription factor-binding sites and enhancer regions, may be analyzed, that could help provide a more detailed insight in future experimentation. Furthermore, more experimentation is required at different exercise intensities/durations to ascertain more definitive genome-wide changes to DNA methylation after chronic exercise in healthy adults.

Overall, in the aerobic exercise field, it remains to be established if any of these DNA modifications are retained after both acute or chronic exercise, even if exercise training ceases. Further, if these modifications are maintained during detraining, how long these modifications are retained for, and if this subsequently influences gene expression when individuals retrain. This notion of epigenetic memory at the DNA methylation level has not been studied in response to aerobic exercise and requires future investigation. However, a recent study investigating repeated chronic aerobic training periods (training, detraining, and retraining) suggested that there was not a retention of gene expression profiles (analyzed via RNAseq) during 9 months detraining in humans, following a prior period of 3 months one-legged aerobic training (Lindholm et al., 2016). Furthermore, following retaining there was no suggestion of a transcriptional (gene expression) memory from earlier training (Lindholm et al., 2016). However, in this paper DNA methylation was not reported and it remains to be determined if a period of detraining less than 9 months evokes either an epigenetic or transcriptional memory. Despite lack of epigenetic data in adult humans after exercise, it has been suggested that skeletal muscle is "programmable" after aerobic exercise between generations. Indeed, it has been demonstrated that the offspring of obese mouse mothers have increased methylation of *Pgc-1α*, glucose transporter type 4 (*Glut4*), cytochrome c oxidase 4 (*Cox4*), and cytochrome C (*CytoC*) with corresponding reductions in gene expression. Importantly, voluntary wheel running exercise (vs sedentary controls with locked running wheels) in the obese mothers could prevent these phenomena in the offspring (Laker et al., 2014). This suggested that epigenetic information can be retained across generations and that the exercise stimulus in the mothers can be epigenetically "remembered" by the muscle of the offspring (Laker et al., 2014). Further, a recent study has demonstrated, following transcriptome wide analysis, that the gene, nuclear receptor subfamily 4 group A member 1 (*Nr4a1*) (a member of the steroid-thyroid hormone-retinoid receptor superfamily where the encoded protein acts as a transcription factor that translocates from the cytosol to mitochondria), in the offspring of high-fat fed mothers was increased

(Kasch et al., 2018). This increase in gene expression was associated with promoter hypomethylation of the same gene. Interestingly, exercise in the offspring could reverse the DNA methylation and gene expression changes and ameliorate the negative effect on insulin sensitivity caused by the maternal high-fat diets (Kasch et al., 2018). Again, this study suggested that there was an epigenetic memory to type 2 diabetes susceptibility in offspring exposed to perinatal high-fat diets, and that exercise could alter this epigenetic memory. In adults, skeletal muscle-derived cells isolated from endurance trained humans (running 50 km/week or had completed 10 marathons—2 within the last year) have been shown to be somewhat resistant to palmitate induced insulin resistance and also displayed improved glucose uptake vs cells from sedentary individuals (Green et al., 2013; Valencia and Spangenburg, 2013), suggesting that muscle cells derived from those who are aerobically trained remember the in vivo niche from which they were derived once isolated in vitro. In future studies, DNA methylation analysis of the cells from both the active and sedentary individuals would be required to identify if there was any epigenetic retention of DNA methylation vs tissue. With the caveat the change in methylation across proliferation and differentiation time course would have been controlled for in the cells, as well as the contribution of methylation from fiber born myonuclei vs resident satellite cells. A study partially addressing this demonstrated that muscle cells isolated from type-II diabetic responders and nonresponders to chronic aerobic exercise (described above) displayed DNA methylation of similar genes at the tissue level (Stephens et al., 2018) suggesting that DNA methylation changes can perhaps be retained from the in vivo tissue to the muscle-derived cells in vitro isolated from type-II diabetics after chronic exercise.

Overall, given the studies described above, future work is required to investigate if DNA methylation changes in the muscle tissue itself are retained in adults if aerobic exercise ceases and therefore how long they are maintained before they are lost. If the scientific community could identify what type, intensity, and frequency of exercise is required to maintain an epigenetic memory of aerobic exercise for extended periods of time, then be this information could be used to optimize training programs for athletes, those with metabolic disease or those previously active individuals recovering from injury (i.e., individuals that are detrained/unconditioned).

10.4 Resistance exercise and DNA methylation in skeletal muscle

DNA methylation changes following resistance exercise were not investigated until very recently where, the first genome wide analysis (450 K CpG sites) of human skeletal muscle only took place in 2014, following 16 weeks of resistance exercise in obese, type-II diabetics (3 days/week, 2–3 sets, 6–8 reps per set, 2 quadricep exercises for site of biopsy with workload increased by 3–5% when participants could perform 10 reps) (Rowlands et al., 2014). Resistance exercise resulted in significantly modified DNA at 555 CpGs, with the majority of sites demonstrating a reduction

(hypomethylation) in DNA methylation (409 CpG sites) vs those that were hypermethylated (146 CpG sites). Pathway enrichment analysis suggested that these significantly modified genes were associated with cellular assembly and organization, cellular development, tissue morphology, and cardiovascular system development and function (Rowlands et al., 2014). Importantly, this was the same study described above that also showed a hypomethylated profile after aerobic exercise across the genome, albeit in distinct pathways than those identified after resistance exercise (Rowlands et al., 2014). Taken together, these studies therefore suggest that both endurance and resistance exercises preferentially hypomethylate the genome and therefore could lead to a more permissive and transcriptionally functional state. In accordance with this, in 2016, a study suggested there was a reduction of global (not gene specific) DNA methylation in leukocytes after 12 weeks chronic resistance exercise (2 days/week, 3–4 sets, 10–12 reps at 70% 1 RM) (Dimauro et al., 2016). Later the same year, the first study to investigate genome-wide DNA methylation after 8 weeks of chronic resistance exercise (3 days/week, 3 sets, 8–12 reps at 80% 1 RM) in leukocytes of healthy males was conducted (Denham et al., 2016). This study demonstrated that resistance exercise evoked significantly enriched DNA methylation profiles in pathways such as axon guidance, diabetes, and immune pathways. They further identified two growth-related genes, growth hormone-releasing hormone (GHRH) and fibroblast growth hormone (FHG1), that demonstrated reductions in methylation with corresponding increases in gene expression. The first genome-wide DNA methylation analysis in human skeletal muscle tissue itself, after resistance exercise in healthy adults was after a period of 10 weeks chronic resistance exercise. However, authors reported nonsignificant changes of less than 10% CpG methylation, while only reporting promoter associated CpGs (Robinson et al., 2017). On the contrary, a study published at a similar time identified that resistance exercise promoted a hypomethylated profile following a short-term high-fat diet (9 days) vs the high-fat diet alone, which demonstrated a hypermethylated profile across the genome (Laker et al., 2017), as analyzed via bisulfite sequencing. It is also worth noting that promoter DNA methylation has recently been associated with reductions in muscle size after disuse atrophy simulated by the inhibition of neural input to the hindlimbs via tetrodotoxin exposure to the common peroneal nerve (Fisher et al., 2017). Where transcriptome analysis identified the most significantly upregulated genes after atrophy, and also highlighted a subset of the same genes that were hypomethylated, including: *Myogenin*, the E3 ubiquitin-protein ligase *Trim63* (also known as muscle ring-finger 1/*MuRF1*), atrogin-1 (*MAFbx*), and cholinergic receptor nicotinic alpha 1 subunit (*Chrna1*). Furthermore, the DNA methylation and gene expression changes were reversed back toward baseline when atrophy ceased and the muscle was allowed to recover back toward baseline levels (Fisher et al., 2017). Suggesting that muscle mass perturbations during atrophy and a return to normal muscle size during recovery were

dynamically linked to changes in DNA methylation and associated with changes in gene expression of the same genes.

Another recent study with comprehensive analysis of genome wide methylation (850,000 CpG sites) after resistance exercise was conducted after both acute and chronic resistance exercise as well as detraining and retraining, using a within subject design (Seaborne et al., 2018a,b). This study demonstrated that there was a significant increase in the number of hypomethylated CpG sites vs baseline biopsies after acute resistance exercise. After chronic exercise, that was associated with increases in lean leg mass, there were also a slightly larger number of hypomethylated vs hypermethylated CpG sites. Interestingly however, the number of both hypomethylated and hypermethylated CpG sites remained stable during detraining when lean muscle mass returned to baseline (preexercise levels). Following retraining, the largest number of hypomethylated CpG sites was observed concomitantly with the greatest amount of lean mass being observed in the participants. This hypomethylation across the genome following chronic resistance exercise was the first to be conducted in healthy individuals, and extends previous observations made using platforms of earlier array technology (Rowlands et al., 2014). The authors identified two key patterns of DNA methylation and associated gene expression across the time course of training, detraining, and retraining (Fig. 10.2) (Seaborne et al., 2018a). The authors then associated these temporal changes with the genes that demonstrated an inverse regulation in gene expression. The first temporal trend included genes, AXIN1, GRIK 2, CAMK4, and TRAF1, that displayed decreased CpG DNA methylation (hypomethylation) with corresponding increased gene expression after chronic training induced hypertrophy (Fig. 10.2). Interestingly, hypomethylation of the same genes CpG sites was maintained even when muscle returned to preexercise levels during detraining. These genes CpG sites then also demonstrated continued hypomethylation into retraining, with a subset of genes demonstrating even greater hypomethylation and enhanced gene expression at this time point. Therefore, these data suggest an epigenetic memory at the DNA methylation level of earlier training induced hypertrophy during detraining that led to even greater gene expression during later retraining. The second temporal trend that included genes, UBR5, RPL35a, HEG1, PLA2G16, and SETD3, that were hypomethylated with enhanced gene expression following training, where methylation and gene expression returned to baseline/preexercise levels during detraining (Fig. 10.2). However, after later retraining, these genes displayed even larger increases in both hypomethylation and gene expression, again suggestive of an epigenetic memory of earlier muscle growth (Seaborne et al., 2018a). This was the first study to identify this type of memory at the DNA level in adult skeletal muscle tissue, with earlier studies suggesting a cellular memory of testosterone inducing hypertrophy in animal models, with retention of myonuclei following prior testosterone treatment, that facilitated more rapid skeletal muscle growth upon later resistance exercise, compared to

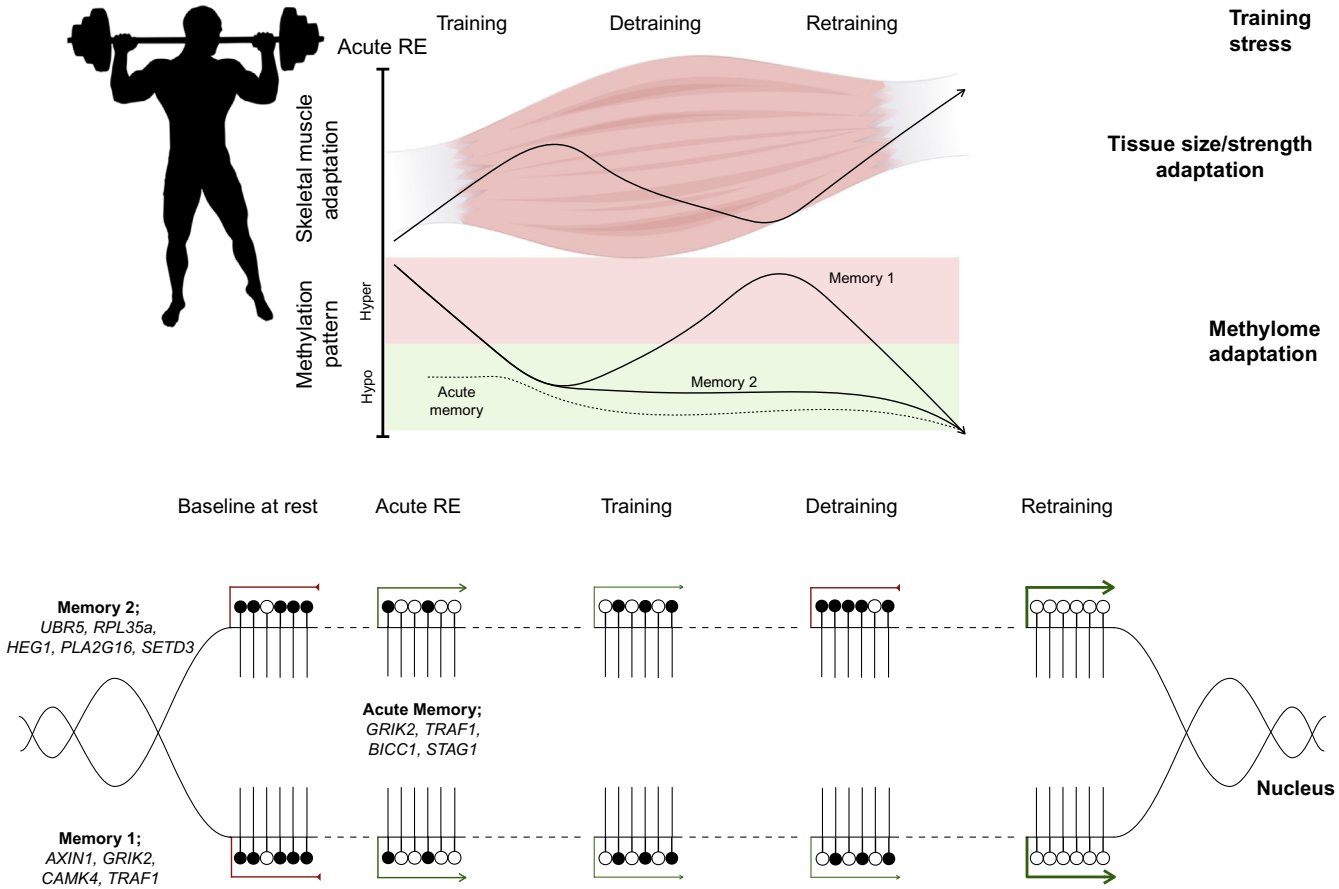

Fig. 10.2 Regulation of DNA methylation after acute resistance exercise (RE), chronic training-induced hypertrophy, detraining (where muscle returns to baseline/preexercise levels) and retraining-induced hypertrophy as recently described in Seaborne et al. (2018a). There were two main patterns of DNA methylation observed and depicted in the schematic representation. **Memory 1**: The first temporal trend included genes, AXIN1, GRIK2, CAMK4, and TRAF1, that displayed significantly decreased DNA methylation (hypomethylation-depicted via the *dark green arrow*) with corresponding increased gene expression after chronic resistance training. Hypomethylation of the same genes was maintained even when muscle returned to preexercise levels during detraining (*light green arrow*). These genes then also demonstrated continued hypomethylation into retraining, with this subset of transcripts demonstrating even greater hypomethylation (*darkest green arrow*) and enhanced gene expression at this time point. Therefore, these data sugges an epigenetic memory at the DNA methylation level of earlier training-induced hypertrophy. **Memory 2:** The second temporal trend that included genes, UBR5, RPL35a, HEG1, PLA2G16, and SETD3, that were hypomethylated with enhanced gene expression following training (*dark green arrow*), where methylation and gene expression returned to baseline/preexercise levels during detraining (demethylation, *red arrow*). However, after later retraining these genes displayed even larger increases in both hypomethylation (*darkest green arrow*) and gene expression, again suggestive of an epigenetic memory of earlier muscle growth (Seaborne et al., 2018a). **Acute memory:** Genes GRIK2, TRAF1, BICC1, and STAG1 were hypomethylated after a single encounter with resistance exercise and that this hypomethylation was maintained throughout training and retraining with the largest hypomethylation and increased gene expression seen at the later retraining time point. This suggested that these genes were acutely sensitive to DNA methylation even after a single bout of exercise, and were then maintained with chronic repeated training and retraining.

relevant controls (Egner et al., 2013; Bruusgaard et al., 2010; Gundersen, 2016). Furthermore, it supports earlier in vitro studies (albeit atrophy not hypertrophy stimuli), where myoblasts have been shown to retain DNA methylation after 30 population doublings when exposed to an early proliferative life encounter with a muscle wasting dose of inflammatory cytokine TNF-alpha (Sharples et al., 2016).

Interestingly, in the above study, mRNA changes of UBR5, RPL35a, HEG1, and PLA2G16 and SETD3 also strongly and positively correlated with the increase in lean muscle after training, detraining, and retraining (Seaborne et al., 2018a). Identifying these genes, in their own right as epigenetically modified hypertrophy genes. In particular UBR5 (an HECT domain E3 ubiquitin ligase), that was hypomethylated after training-induced hypertrophy, and its gene expression were positively correlated with increasing lean leg mass after training and retraining in humans, and have been more recently extensively characterized and confirmed to be important in skeletal muscle hypertrophy, atrophy, and recovery from atrophy across mammalian species in vivo and in vitro (Seaborne et al., 2019). In this most recent study, the authors demonstrated that, in alternate fashion to well-known muscle-specific E3 ligases (MuRF1/MAFbx), UBR5 was elevated at the gene expression level during recovery from atrophy in rats, acute anabolic mechanical loading in mouse bioengineered muscle, after hypertrophy in rats and mice in vivo, as well as after human muscle cell differentiation in vitro (Seaborne et al., 2019). Furthermore, in humans in vivo, the A alleles of the UBR5 rs10505025 and rs4734621 SNPs that affect the expression of the UBR5 gene, according to GTEX (Battle et al., 2017), were strongly associated with larger fast-twitch muscle fibers and strength/power performance versus endurance status in athletes. Importantly, in relation to exercise and DNA methylation, an increase in UBR5 gene expression was observed after acute mechanical loading of mouse bioengineered muscle (1.58-fold) (Seaborne et al., 2019) supporting previous work in humans and demonstrating a similar fold change in UBR5 gene expression (1.71-fold) after a single bout of resistance exercise in the skeletal muscle tissue of humans (Seaborne et al., 2018a). While gene expression was significantly elevated after the acute loading of bioengineered muscle, the study only identified one out of six CpG sites located in UBR5 promoter to be nonsignificantly hypomethylated (Seaborne et al., 2019). This, however, confirmed previous studies where there was no change in UBR5 DNA methylation in skeletal muscle tissue after acute resistance exercise in humans (Seaborne et al., 2018a). Despite this, as alluded to above, significant hypomethylation of UBR5 was demonstrated to occur after repeated/chronic training and retraining-induced hypertrophy in humans (Seaborne et al., 2018a), and in the later study it has also been demonstrated that hypertrophy (14% increase in mass) in rat muscle after chronic intermittent electrical simulation evoked mean reductions in promoter UBR5 methylation (hypomethylation), albeit this did not quite reach statistical significance (Seaborne et al., 2019). Despite this, collectively

with the data in humans these studies point toward repeated and chronic loading/exercise stimuli being required to cause promoter UBR5 hypomethylation, yet with gene expression of UBR5 elevated in response to both acute and chronic exercise stimuli. Also, given previous work suggesting that hypomethylation of UBR5 is retained during detraining after a period of training-induced atrophy (Seaborne et al., 2018a), it is possible that once hypomethylation occurs in UBR5 after chronic exercise it may be relatively stable, and these changes can be retained over longer periods even when hypertrophic stimuli are removed, in relation to retention of DNA methylation over longer periods. Seaborne et al. (2018a) also demonstrated that genes GRIK2 and TRAF1, as well as BICC1 and STAG1 were hypomethylated after a single encounter with resistance exercise and that this hypomethylation was maintained throughout training and retraining (23 weeks later) with the largest hypomethylation and increased gene expression seen in later retraining time point (Fig. 10.2). This suggested that these genes were acutely sensitive to DNA methylation even after a single bout of exercise, and were then maintained with chronic repeated exercise. While this study was fairly comprehensive, the authors used a targeted approach for gene expression analysis, based on the most significantly modified and most frequently occurring methylated genes at the genome-wide DNA level. While this is a valid approach it did not allow corroboration of the significantly enriched pathways at the methylome level to be overlapped with those across the entire transcriptome. However, the same group (Turner et al., 2019) have recently undertaken a large-scale bioinformatic analysis of pooled transcriptome data after acute and chronic resistance exercise in the majority of studies conducted to date (using publicly available transcriptome data sets) (Turner et al., 2019), and overlapped this with the recent methylome changes described after acute and chronic training, detraining, and retraining (Seaborne et al., 2018a,b). In this work, it was demonstrated that 866 genes were upregulated after acute resistance exercise with 270 of these genes being hypomethylated, whereas 936 genes were downregulated and 216 were hypermethylated (Turner et al., 2019). After chronic resistance exercise, 2018 genes were upregulated with 592 identified as hypomethylated, and 430 genes downregulated with only 98 hypermethylated, again demonstrating that more genes were hypomethylated vs hypermethylated, particularly after chronic resistance exercise. After pathway analysis across both the pooled transcriptome and methylome data after acute and chronic RE, the analysis also identified that "cancer pathways" (according to KEGG pathway classifications) were significantly enriched in both analyses. The genes in this pathway classification were "pro-growth" genes, where specifically in skeletal muscle their functions include: matrix and actin structure/function and remodeling, mechano-transduction (including PTK2/Focal Adhesion Kinase/FAK and Phospholipase D—following chronic resistance exercise analysis only), TGF-beta signaling, and protein synthesis (GSK3B after acute resistance exercise only). This work also identified that 51 genes were up/downregulated in

the pooled transcriptomic studies after acute and chronic resistance exercise, and were also significantly modified at the DNA methylation level in all conditions of acute resistance exercise, chronic training, detraining, and retraining. With five genes demonstrating an epigenetic memory profile at the DNA methylation level, where the hypomethylation was retrained even during detraining when muscle returned to pre-exercise levels. In particularly, the gene Filamin B (FLNB) was significantly upregulated at the gene expression level in the acute and chronic transcriptome analysis and was significantly modified at the DNA methylation level after acute, chronic resistance exercise, detraining, and retraining (Turner et al., 2019). Filamin B gene encoded at the protein level is involved in connecting the cell membrane constituents to the actin cytoskeleton. Filamin A and C have been investigated following endurance and resistance exercise, respectively (Deshmukh et al., 2006; Ulbricht et al., 2015), and associated with autophagy (Ulbricht et al., 2015). However, there is limited information for the role of FLNB in skeletal muscle or exercise adaptation. Therefore, further gene expression analysis in Turner et al. (2019) of the samples derived from Seaborne et al. (2018a,b), confirmed FLNB was increased at the gene expression level after acute and chronic resistance exercise and remained elevated after detraining and retraining where the gene remained as hypomethylated even during exercise cessation (detraining) (Turner et al., 2019). However, it is worth noting that increases in FLNB were only significant after acute resistance exercise. Despite this, and given the associated sustained hypomethylation, the authors suggested that this gene requires further mechanistic investigation as to its role in skeletal muscle anabolism, hypertrophy, and epigenetic memory (Turner et al., 2019).

Finally, future studies are now required to distinguish the role of epigenetics in muscle memory after muscle wasting encounters following disuse after injury or inactivity, and if elderly individuals become more susceptible to repeated muscle wasting encounters. Importantly, with the above knowledge supporting epigenetic memory at the DNA level in healthy adults after muscle growth, it would be plausible to hypothesize that exercise could reverse some of the epigenetic alterations in skeletal muscle with age. Indeed, a study has demonstrated DNA is hypermethylated at the genome-wide level in aged skeletal muscle tissue compared to young adult tissue (Zykovich et al., 2014). With approximately 500 different sites (DNA methylation "signature") that could identify if the tissue was young or aged (Zykovich et al., 2014). This suggests that in skeletal muscle with age, DNA methylation is accumulated or retained across the genome. By contrast, the studies reviewed above suggest that both aerobic and resistance exercise seem to hypomethylate genes across the genome, suggesting a potential mechanism behind exercise and its anti-aging effects. However, to date, genome-wide DNA methylation after exercise in the skeletal muscle of elderly vs young adults has not been investigated, with further work required to elucidate these hypothesizes.

10.5 Conclusion

Alterations in DNA methylation are associated with the regulation of metabolic gene expression after aerobic exercise and genes associated with muscle mass regulation after resistance exercise. Skeletal muscle also displays an epigenetic memory at the DNA methylation level following previous resistance exercise and muscle growth encounters. Future research into epigenetics of exercise performance and adaptation could provide key information to enhance performance or improve recovery from injury in athletes, and optimize exercise to reduce metabolic disease and enable healthy ageing.

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CHAPTER ELEVEN

Genes and individual responsiveness to exercise-induced fat loss

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11.1 Introduction

Regular physical activity has significant benefits for health, including reduction of the risk of cardiovascular diseases, hypertension, metabolic diseases (e.g., type 2 diabetes), certain forms of cancer, fatty liver disease, pulmonary diseases, hormonal disturbances, osteoarticular diseases, and psychiatric illness. Additionally, properly selected exercises are key component of the total daily energy expenditure and as such contribute to improve body composition and help control weight (Rankinen and Bouchard, 2008). According to the World Health Organization (WHO), the number of people with overweight and obesity is increasing rapidly worldwide and is described as an epidemic, consequently, the prevention of weight gain is very important public health problem (Bray and Bellanger, 2006).

An accumulation of additional amounts of triglycerides (TGL) in adipose tissue is a consequence of a chronic excess of energy intake compared to energy expenditure. The imbalance of energy homeostasis can be affected by both caloric intake and physical activity, which may be dependent on developmental, behavioral, and environmental factors (Rank et al., 2012). Additionally, genetic factors play a key role in the regulation of body weight, since there are genes involved in the regulation of energy expenditure, appetite, lipid metabolism, adipogenesis, thermogenesis, and cell differentiation (Deram and Villares, 2009). The reported heritability of obesity varies depending on the phenotype studied, but tends to range from 31% to 90% (Min et al., 2013). In most individuals, the genetic background of obesity is complex and multifactorial, involving the interaction of numerous genes and gene-environment interactions. As with other complex phenotypes, rare examples of mono- and oligogenic causes for obesity that serve as models for understanding the difficult hormonal and neural networks that regulate energy homeostasis have been reported (Walley et al., 2006; Chung, 2012).

Sports, Exercise, and Nutritional Genomics
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However, the large number of single nucleotide polymorphisms (SNPs) identified by genome-wide association studies (GWASs) and candidate gene studies, appeared to explain only 2%–4% of the obesity status (El-Sayed Moustafa and Froguel, 2013). As a result, individual genetic variations cannot account for the present increasing obesity epidemic. As the environment conditions become increasingly obesogenic, measurable variation in individual obesity levels becomes apparent. Such differences in individual phenotype at these extremes of adiposity likely reflect allelic variation at genes that affect energy intake and expenditure (Chung, 2012).

Decades of physiological research in physical activity has resulted in relatively good knowledge of the functional response of human body to exercise. Although the physiological reactions after regular exercises are quite well described, the genetic background of these reactions still remains mostly unknown (Leońska-Duniec, 2013). The process of exercise-induced adaptation in human body involves a number of signaling mechanisms, initiating replication of specific DNA sequences, enabling its following translation, and finally generating new proteins. The physiological effects of these adaptations are determined by volume, intensity, and frequency of physical activity (Coffey and Hawley, 2007). It is well known that individuals vary in their responses to similar training: from a lack of adaptive response to extreme overload. Current studies have shown that people with the same genotypes respond similarly to exercises in comparison to those with different genotypes, indicating that some genes play a key role in determination of individual differences in response to physical activities. An understanding of the genetic determinants will allow to clarify the criteria of physical activities for individuals. In the future, this knowledge should help to identify persons who are expected to respond well or poorly to exercise, thus making training programs much more efficient (possibility of accurate prediction of the training results including weight loss and improve health) and safer (early prevention of possible overload, injuries, cardiomyopathies, sudden death, etc.) (Leońska-Duniec, 2013).

At first, studies performed in families, adoptees, and twins have clearly shown there is a genetic contribution to obesity. However, they do not offer insight into the specific gene variation underlying heritable traits. Currently, new technologies allow scientists to study the structure and function of genes at a molecular level. The search for genetic markers associated with obesity was firstly carried out using candidate-gene association studies or linkage analysis. Although these were promising methods in the discovery of genes for syndromic and monogenic obesity, the success of finding genes for common obesity susceptibility was limited, with very few reproducible results (Rankinen et al., 2006; Herrera and Lindgren, 2010). The considerable advances in the discovery of genetic loci that are linked with obesity-related traits were made in 2007 (Frayling et al., 2007; Scuteri et al., 2007). This was possible through analytical and technical progress allowing for GWASs (Herrera and Lindgren, 2010). GWASs allow for the analysis of the whole genome polymorphic sites to link genetic markers, usually SNPs, to

physiological traits (Kim et al., 2011). At present, more than 700 genes and chromosomal regions have been described to take part in body weight and energy metabolism regulation, whereas gene-environment interaction in relation to weight change has been studied less frequently (Locke et al., 2015).

Numerous evidences suggest that genetic factors play a significant role in how individuals respond to exercise. As some specific genetic factors for body weight have recently been identified, there is increasing interest in whether and how genetic susceptibility might interact with lifestyle factors such as physical activity (Li et al., 2010; Ahmad et al., 2013). This chapter summarizes the current evidence on the role of the gene variants on the characteristics and range of the body's adaptive response to training. We studied the most reliable candidate genetic markers that are involved in energy balance pathways and body composition changes in response to training programs.

11.2 Gene variants and exercise-induced fat loss

11.2.1 *FTO* gene

The first described obesity-susceptibility gene, with the largest influence on higher body mass index (BMI) to date, was the fat mass and obesity-associated gene (*FTO*) (Frayling et al., 2007; Scuteri et al., 2007). Recently, studies concerning the relationship between *FTO* and weight have been frequently replicated, not only for BMI, but also for obesity risk, body fat percentage, waist circumference, type 2 diabetes, and other types of obesity. Subsequently, these associations are replicable across different age groups, as well as multiple ethnic populations (Loos, 2012). Currently, a common *FTO* A/T polymorphism (rs9939609) is one of the most often investigated genetic variant in the context of genetic conditioning for a predisposition to body weight excess.

The human *FTO* gene is located in chromosome region 16q12.2 (Frayling et al., 2007), and the product of the gene is a nuclear protein—2-oxoglutarate (2-OG) Fe(II) dependent demethylase (Loos and Yeo, 2014). The up-to-date results establish that the enzyme is able to remove methyl groups from DNA and RNA nucleotides in vitro with the highest affinity for single stranded RNA molecules (Almén et al., 2012; Loos and Yeo, 2014). It was suggested that the *FTO* gene can influence the activity of pathways controlling daily food intake, and what is more nutrient preference (Loos and Yeo, 2014).

The *FTO* A/T polymorphism is located in the first intron of the gene, which is related with an enhanced risk of excessive weight gain, increasing the risk by 20%–30%. It has been revealed that carrier status of one or two copies of the risk (A) allele is related with increases in body mass of average 1.2 and 3.0 kg, respectively (Frayling et al., 2007). Numerous studies have reported that the *FTO* effect on obesity-related traits is reduced by approximately 30% in physically active compared to sedentary adults (Li et al., 2010; Kilpeläinen et al., 2011; Ahmad et al., 2013; Loos and Yeo, 2014).

In other studies, the effect size of *FTO* variants is up to 80% lower in physically active individuals (Rampersaud et al., 2008; Vimaleswaran et al., 2009). However, not all studies have demonstrated the gene × physical activity interaction (Hakanen et al., 2009; Jonsson et al., 2009; Lappalainen et al., 2009). Although, Leońska-Duniec et al. (2018a) have confirmed the association between the *FTO* A/T polymorphism and increased BMI, none of the examined obesity-related parameters changed significantly across the *FTO* genotypes during a 12-week training program (Leońska-Duniec et al., 2018a).

11.2.2 *MC4R* gene

The melanocortin-4 receptor gene (*MC4R*) encodes a 332-amino-acid, which belongs to a family of seven trans-membrane G-protein-coupled receptors (GPCRs). The protein is well-known major regulator of food intake and energy expenditure. Polymorphisms within the *MC4R* coding region have been described to be related with obesity in humans (Hebebrand et al., 2010). In addition, variants outside of the coding region probably influence its expression and have been linked with a predisposition to excess of body weight (Evans et al., 2014). GWAS conducted in Caucasian revealed that the variant rs17782313 (C/T polymorphism), mapped 188 kb downstream of the *MC4R* (Loos et al., 2008), also shows a strong connection with obesity-related traits (Xi et al., 2012). This association has been confirmed in multiple populations including children, adolescence, and adults (Loos, 2012; Xi et al., 2012).

The risk (C) allele is connected with an increased intakes of total energy and dietary fat, and as a result higher prevalence of obesity (Qi et al., 2008). Each copy of the C allele is linked with an increase in BMI of ~0.22 kg/m^2 in adults (Loos et al., 2008). What is more, the risk allele was also associated with average 14% enhance risk of type 2 diabetes (Qi et al., 2008). It has been described that effect of the gene on obesity-related traits may be reduced by living a physically active lifestyle. Li et al. (2010) genotyped 12 SNPs in obesity-susceptibility loci including rs17782313 in a group of 20,430 European participants and showed that genetic predisposition to increased BMI and obesity is attenuated by a physically active lifestyle (Li et al., 2010). However, other study did not show association between the polymorphism and selected body composition measurements in 242 participants undergoing a 9-month lifestyle intervention (Haupt et al., 2009). In a study performed on 111,421 adults of European descent, Ahmad et al. (2013) analyzed 12 loci connected with obesity-related traits and also did not reveal evidence of the polymorphism rs17782313 × physical activity interactions (Ahmad et al., 2013). Additionally, Leońska-Duniec et al. (2017) could not notice the near *MC4R* C/T polymorphism × physical activity interaction in a group of 201 Polish women taking part in 12-week training program (Leońska-Duniec et al., 2017).

11.2.3 *PPARG*, *PPARD*, and *PPARGC1A* genes

The peroxisome proliferator-activated receptors genes (*PPAR*) are frequently investigated genetic markers in the context of athletic predisposition and health-related fitness phenotypes due to the multiple physiological roles of proteins encoded by them (Maciejewska-Karłowska, 2013). PPAR proteins are lipid-activated nuclear receptors which are the member of the nuclear hormone receptor superfamily (Desvergne and Wahli, 1999). The transcriptional activity of PPARs is mediated by PPAR retinoid X receptor (RXR) heterodimers which bind to specific DNA sequence elements termed PPREs (PPAR response elements) in their target genes' regulatory region. The major role of PPARs is the transcriptional regulation of proteins involved in lipid and carbohydrate metabolism. Additionally, PPARs effect expression of genes active in vascular biology, tissue repair, cell proliferation, and differentiation (Yessoufou and Wahli, 2010). Three PPAR isotypes have been described so far, which exhibit a different tissue distribution, functions, and to some extent different ligand specificities: (i) PPARα encoded by the *PPARA* gene located on chromosome 22, (ii) PPARδ (also called PPARβ) by the *PPARD* gene on chromosome 6, and (iii) PPARγ by the *PPARG* gene on chromosome 3 (Maciejewska-Karłowska, 2013).

Peroxisome proliferator-activated receptor gamma, coactivator 1 alpha (PGC1), encoded by the *PPARGC1A* gene, is a transcriptional coactivator of the PPAR superfamily. This protein interacts with PPARγ which enables its interaction with many others transcriptional factors. PGC1 is involved in mitochondrial biogenesis, glucose utilization, fatty acid (FA) oxidation, thermogenesis, gluconeogenesis, and insulin signaling. Peroxisome proliferator-activated receptor gamma, coactivator 1 beta (PGC1), encoded by the *PPARGC1B* gene, together with the *PPARGC1A* gene, encodes homologous proteins that, through nuclear transcription factor coactivation, regulate adipogenesis, insulin signaling, lipolysis, mitochondrial biogenesis, angiogenesis, and hepatic gluconeogenesis (Franks et al., 2014).

The *PPARs* and their transcriptional coactivators' gene polymorphisms due to their role in lipid and carbohydrate metabolism are frequently described as genetic markers which influence obesity and other disease-related phenotypes obesity. Currently, especially *PPARG*, *PPARD*, and *PPARGC1A* genes are considered in the context of their potential impact on functional response of the human body to exercise.

Zarebska et al. (2014) have checked if body mass changes observed in physically active participants will be modulated by the *PPARG* Pro12Ala (rs1801282) genotype. The results suggest that *PPARG* genotype may modulate training-induced body mass measurements changes: after completion of the training program Pro12/Pro12 homozygotes were characterized by a greater decrease of body fat mass measurements in comparison with 12Ala allele carriers. These results indicate that the *PPARG* 12Ala variant may

weaken the training-induced positive effects on body mass measurements (Zarebska et al., 2014). Other interventional studies have shown that diet and physical activity connection on fasting insulin differ between *PPARG* Pro12Ala genotypes. The beneficial additive results of exercise and healthy diet were noticed only in homozygotes for the Pro12 allele. Meanwhile, in 12Ala allele carriers association between diet and exercise were more complicated and the change in fasting insulin level was only attenuated when both exposures of diet and activity were simultaneously elevated (Franks et al., 2004).

The studies of *PPARD* polymorphisms have been mainly focused on three SNPs: rs2267668 with its *locus* in intron 3, rs2016520 with its *locus* in the 5′ untranslated region of exon 4, and rs1053049 with its *locus* in the 3′ untranslated region of exon 9. Leońska-Duniec et al. (2018b) correlate the distribution of genotypes, alleles, and haplotypes described in mentioned above *PPARD* polymorphisms in female participants engaged in a 12-week training program. With reference to *PPARD* rs2267668 genotypes, there were two significant genotype × training interactions in which a greater decrease of cholesterol (Chol) over training was observed in the rs2267668 G allele carriers and a significant increase of TGL levels in the AA homozygotes. Moreover, carriers of a rs2016520 *PPARD* C allele exhibited a significant decrease in Chol from training with accompanying decreases in TGL. There was also overrepresentation of *PPARD* rs1053049 TT homozygotes in the group with higher posttraining TGL levels. Moreover (rs2267668/rs2016520/rs1053049) G/C/T haplotype displayed smaller posttraining body mass decrease, suggesting that harboring this specific G/C/T haplotype is unfavorable for achieving the desired training-induced body mass changes. On the other hand, the G/C/C haplotype was significantly associated with posttraining increase in fat free mass (FFM) and with lower levels of Chol as well as TGL as observed in the blood of the participants in response to applied training (Leońska-Duniec et al., 2018b).

Associations of *PPARGC1A* polymorphisms with a range of cardiovascular and metabolic traits, including type 2 diabetes, insulin resistance, glucose concentrations, dyslipidaemia, obesity, and aerobic fitness, have been reported, as have interactions between gene variants and lifestyle factors including regular physical activity (Franks et al., 2014). The *PPARGC1A* rs8192678 Gly/Gly genotype has been associated with greater increases of an individual's anaerobic threshold, slow muscle fibers, mitochondrial activity, as well as reduction of total Chol and low-density lipoprotein (LDL) after lifestyle intervention including regular aerobic training than *PPARGC1A* rs8192678 Ser allele carriers. Additionally, *PPARGC1A* rs8192678 Ser allele carriers had no response in changes of slow twitch muscle fibers, total Chol and LDL after aerobic training (Stefan et al., 2007; Steinbacher et al., 2015; Tobina et al., 2017). These observations led to the suggestion that the rs8192678 Gly allele may be a key element associated with the efficiency of aerobic metabolism (Stefan et al., 2007).

11.2.4 *LEP* and *LEPR* genes

Leptin, an adipocyte-derived hormone, which plays key role in regulating appetite by its inhibitory effects on food intake and increases in energy expenditure by stimulating metabolic rate and physical activity to maintain energy balance (Lenard and Berthoud, 2008). Leptin signaling is mediated by its specific receptor, a single transmembrane protein which belongs to class I cytokine receptor family (Considine et al., 1996). Leptin acts as an afferent signal in a negative feedback loop by binding to leptin receptor regulating the size of adipose tissue (Sahu, 2004).

Several polymorphisms of both genes coding leptin (*LEP*) and leptin receptor (*LEPR*) have been studied in various populations for their potential association with obesity. That common variants also may modify the effects of regular physical activity on various obesity-related traits such as glucose homeostasis (Lakka et al., 2004). Among these SNPs, the leptin concentration is affected by the LEP A19G polymorphism (rs2167270) of the untranslated region of exon 1. The genotype GG is connected with significantly lower leptin concentrations in comparison with the genotype AA (Hager et al., 1998). In a study performed on 242 European-derived participants, Walsh et al. (2012) revealed that homozygous for the G allele may be receiving additional health benefits as a result of expending more energy in vigorous intensity physical activity due to their genetic predispositions than carriers of the A allele (Walsh et al., 2012). Additionally, Leońska-Duniec et al. (2018c) have suggested that the *LEP* rs2167270 genotype may contribute to improvements in glucose homeostasis in response to a 12-week aerobic training program. A training-related decrease in plasma glucose concentration in the *LEP* AG heterozygotes differed significantly from the change in the AA and GG homozygotes (Leońska-Duniec et al., 2018c).

Variants of *LEPR* have also been described to influence leptin receptor activity. One of them is *LEPR* A668G (rs1137101), which is located in exon 6, a supposed leptin-binding region, and as a result impacts binding capacity of leptin receptor to leptin (Chagnon et al., 1999). The G allele has been connected with greater muscle volume than participants with the AA genotype and greater subcutaneous fat volume response to a resistance training program (Walsh et al., 2012). Additionally, the significant interaction between regular physical activity and *LEPR* rs1137101 for LDL level has been described. As opposed to AG and GG, AA homozygotes have demonstrated a training-related decrease in LDL plasma levels (Leońska-Duniec et al., 2018c).

11.2.5 *ADIPOQ* gene

One of the most frequently investigated adipose tissue-derived hormones is adiponectin, which is an important antiinflammatory and insulin-sensitizing peptide, which promotes lipid oxidation in tissues such as skeletal muscle and liver (Yamauchi et al., 2001, 2002).

The hormone also directs antiatherosclerotic properties, as it strongly inhibits expression of adhesion molecules and growth factors (Kadowaki and Yamauchi, 2005). In humans, adiponectin is encoded by the *ADIPOQ* gene which is located on chromosome 3q27 (Takahashi et al., 2000).

Nowadays, among genetic variants of the *ADIPOQ* gene which have been described in the context of genetic conditioning for a predisposition to obesity in some ethnic populations, +276 G>T SNP (rs1501299) and −11377 G>C SNP (rs266729) are the most frequently investigated polymorphisms associated with serum levels of adiponectin (Enns et al., 2011). The studies suggest that the *ADIPOQ* genotypes analyzed individually or in combination can modulate training-induced body mass measurement changes: after the training program, carriers of the rs1501299 T allele and rs266729 C allele were characterized by a greater reduction in fat mass and body mass compared with G allele carriers. Moreover, *ADIPOQ* polymorphisms were associated with changes in lipid profile in response to training. From this evidence, it could be concluded that rs1501299 G and rs266728 G variants may be considered as a disadvantageous factor in the context of training-induced effects on body mass traits (Kyriakou et al., 2008; Passariello et al., 2010; Leońska-Duniec et al., 2018d).

11.2.6 *ADRB2* and *ADRB3* genes

The proteins encoded by the β2-adrenergic receptor (*ADRB2*) and the β3-adrenergic receptor (*ADRB3*) genes belong to the family of β-adrenergic receptors, which mediate catecholamine-induced activation of adenylate cyclase through the action of G proteins. They are located in adipose tissue and involved in energy homeostasis through the mediation of the both lipolysis and thermogenesis rate. Thus genes encoding these receptors are interesting candidates for explaining part of the genetic predisposition to obesity in humans (Park et al., 2005). Additionally, many studies have shown that variation within *ADRB* genes contributes to interindividual changes of body composition in response to regular physical exercise (Sakane et al., 1997; Corbalán et al., 2002; Shiwaku et al., 2003; Masuo and Lambert, 2011; Leońska-Duniec et al., 2018e).

The ADRB2 is a major lipolytic receptor in adipocytes and genetic polymorphisms in the gene may reduce lipolysis and predispose to obesity. The most frequent variants result in amino acid changes investigated in relation to obesity are at codon 16 (Arg16Gly, rs1042713) and codon 27 (Gln27Glu, rs1042714). The Gly16 allele has been associated with lower receptor density and hence reduced efficiency in comparison with the Arg16 allele, which may influence the propensity to higher BMI (Chou et al., 2012). In a study of overweight men participated in a 24-month weight loss program consisting of low-calorie diet and everyday aerobic exercise showed a higher frequency of the Gly16 allele in men resistant to weight loss and those who regained body weight after successful initial weight loss at 6 months (Masuo et al., 2005). Numerous studies have also shown that the

Glu27 allele may limit *ADRB2* down-regulation and thus affect body weight (Lange et al., 2005). Corbalán et al. (2002) described that women who were more active during their free time and were carriers of the Glu27 allele had higher body weight compared to noncarriers, suggesting that these women may be more resistant to losing weight (Corbalán et al., 2002).

The ADRB3 is the key receptor mediating catecholamine-stimulated thermogenesis in adipose tissue (Emorine et al., 1989). In humans low ADRB3 activity could promote obesity through decreased function in adipose tissue. A Trp64Arg (rs4994) variant in codon 64 of this gene has been associated with a tendency toward excess of body weight, insulin resistance, and type 2 diabetes (Clément et al., 1995; Widén et al., 1995). Many studies have shown increased BMI (average 0.28 kg/m^2) in carriers of the Arg64 allele only among sedentary participants, but not in physically active subjects, where genotypic differences in BMI were not found (Fujisawa et al., 1998; Kurokawa et al., 2001; Marti et al., 2002). Other studies have shown that women with the Arg64 allele who participated in lifestyle intervention combining exercise and low-calorie diet lost less weight than women without the allele, suggesting that the Arg64 allele is connected with difficulty in losing weight through diet and training program (Sakane et al., 1997; Shiwaku et al., 2003). However, Phares et al. (2004) have shown that the Arg64 carriers experienced a great loss of fat mass and trunk fat following 24 weeks of aerobic exercise training compared to noncarriers and demonstrated an opposite allelic response to exercise.

11.2.7 *ACSL1* gene

Mouse studies have revealed that Acyl-CoA synthetase (ACSL) acts to convert long chain FA to acyl-CoA, and thus enhancing FA transport across the plasma membrane and providing substrates for most downstream pathways that metabolize FA. ACSL1 is one of five ACSL isoforms, each encoded by a separate gene. As such, *ACSL1* is highly expressed in tissues that undergo high rates of metabolism, including muscle, adipose tissue, and liver cells (Mashek et al., 2006). The ACSL1 deficiency has been shown to reduce FA oxidation and increase glucose utilization (Ellis et al., 2010), negatively impacting metabolic health (Li et al., 2009). The current studies provide evidences in humans of *ACSL1* SNPs associated with fasting glucose, diabetes, subclinical atherosclerosis and suggest links among these traits and acyl-CoA synthesis (Manichaikul et al., 2016).

A SNP in this gene, rs9997745, has been shown to modify the risk of metabolic disease (Phillips et al., 2010). A second SNP, rs6552828, explained 6.1% of the variance in VO_2max changes following aerobic training in the HERITAGE Family Study (Bouchard et al., 2011). In the latest not published study, a third SNP, rs116143768, was showed a genome-wide significant association ($P = 1.18 \times 10^{-9}$) with fat loss following a 12-week aerobic training, with rare T allele carriers losing 31.4% of their fat

mass, and CC homozygotes losing only 3.8%. From this evidence, it could be concluded that T allele may be considered as a favorable factor in the context of training-induced effects on body mass traits. However, more experimental studies are needed to establish the association between the *ACSL1* SNPs and physical activity.

11.2.8 *INSIG2* gene

Insulin-induced gene 2 (*INSIG2*), located on chromosome 2q14, has been functionally linked to lipid metabolism, mostly due to its role in endogenous Chol and FA synthesis feedback inhibition. An endoplasmic reticulum membrane bound protein, encoded by *INSIG2* gene, inhibits the proteolytic activation of sterol response element-binding proteins (SREPs) in response to Chol or insulin (Gong et al., 2006).

Polymorphisms within *INSIG2* have been associated with BMI, in particular, rs7566605 in the promotor region of this gene was linked to baseline subcutaneous fat mass in females and BMI (Lyon et al., 2007; Orkunoglu-Suer et al., 2008). In previous researches, the C allele was associated with a gain in subcutaneous fat mass following resistance training in males (Orkunoglu-Suer et al., 2008), and following a generalized health 1 year intervention in obese children (Reinehr et al., 2008). The results of the latest not published study performed on 116 Caucasian women fit with the previous research, as CC genotypes was associated with an increase in BMI in response to a 12-week aerobic training. These data suggest that exercise training may indeed lead to poorer health outcomes in *INSIG2* C carriers, in that even limited localized exercise may trigger an increase in adiposity (Orkunoglu-Suer et al., 2008).

11.2.9 *FABP2* gene

Fatty acid-binding proteins (FABPs) are small intracellular polypeptides found in many tissues, involved in FA transfer and metabolism (Hsu and Storch, 1996) and encoded by a family of different genes. The fatty acid-binding protein 2 gene (*FABP2*) is located in the 4q28-4q31 chromosomal region, and encodes the intestinal form of FA-binding protein (IFABP), and is only expressed in the small intestine epithelial cells (enterocytes). IFABP protein plays a key role in the facilitation of cellular uptake and intracellular transport of dietary long-chain FA (Hertzel and Bernlohr, 2000; Furuhashi and Hotamisligil, 2008).

Among the well-known SNPs associated with adiposity is the G to A polymorphism (rs1799883) of codon 54 in exon 2 results in the exchanges an alanine (Ala) in the small helical region of the protein for threonine (Thr). Various studies have suggested that this substitution is a functional mutation, which results in modified FA absorption, it could in turn affect the lipid metabolism and correlate with cardiovascular disease (CVD) risk (Baier et al., 1995). Because of the role of the Ala54Thr polymorphism of the *FABP2* gene, it is considered that can modify interindividual responses to a particular diet and training program (Takakura et al., 2005; de Luis et al., 2006). Takakura et al. (2005) have

showed that subjects with Ala/Thr and Thr/Thr genotype, adjusted resting metabolic rate (RMR) was significantly lower than the subjects with Ala/Ala genotype. Subjects with the Thr54 allele showed significantly greater waist circumference after diet and exercise therapy than subjects with Ala/Ala genotype (Takakura et al., 2005). This result may be supported by other studies, which described that carriers of the Thr54 allele, compared to Ala/Ala homozygotes, failed to have a significant reduction in fat mass, LDL levels, and leptin levels after a lifestyle modification program consisting of diet and physical activity (de Luis et al., 2006).

11.3 Conclusions

Obesity is a multifactorial abnormality which have well-confirmed strong genetic basis but requires environmental influences caloric intake and physical activity to demonstrate. Numerous studies have shown the role of lifestyle including exercise and dietary factors in weight control (Qi and Cho, 2008). However, the problem is in defining the genes and polymorphisms related to obesity, and describing by which mechanisms they exert their effect. In view of the fact that DNA variants do not completely explain the heritability of obesity, more studies with appropriate designs and statistical power should be undertaken using the latest genomics methods in sequencing and genotyping, combined with epigenomics, transcriptomics, proteomics, and metabolomics (Qi and Cho, 2008; Pérusse et al., 2013). Based on the literature, we speculate that in the near future, more studies should be also focused on identifying genetic markers referring to other obesity-related traits, for example, resistance to stress and pain, increased appetite and nutrient preference, as well as temperament.

Another important question is the role of the gene variants on the characteristics and range of the body's adaptive response to training. It is well known that the adaptive changes in the human body in response to regular physical exercises show great individual variance. Consequently, losing weight and changes in obesity-related traits in response to training programs may be more effective for some genotypes than others. However, the genetic background of these reactions still remains mostly unknown. One of the major aims of exercise genomics is to finally be able to define molecular markers, which by themselves or in combination with other biomarkers would make it possible to predict the benefits from exercise program or physically active lifestyle. Understanding the genetic background of physiological processes would have an enormous impact not only on individualization of exercise programs to be more efficient and safer, but also better recovery, traumatology, medical care, diet, supplementation, and many other areas (Pérusse et al., 2013). Currently, some studies have tried to answer these questions, however, they still represent only the first steps towards a understanding of the genetic factors that influence obesity-related traits, and gene variants × physical activity interactions, so continuing researches are necessary.

Nevertheless, the search for genetic markers of functional response of human body to physical activities is very complicated and obtained results may be contradictory. There could be several reasons for this inconsistency: (i) heterogeneity between study populations, (ii) differences in daily food intake and nutrient preference, (iii) discrepancy in the volume, intensity and frequency of exercises and in methods of measuring physical activity, (iv) relatively small size of study group what may not possess sufficient statistical power for meaningful analysis and interpretation. The major problem in this kind of research is the experiment organization consisting of conduction of regular physical activity, food intake control, examination of genotype distribution, and measurement of body composition, physiological, and biochemistry parameters before and after the realization of training program. As a consequence, there is a limitation of the number of people participating in lasting few weeks or even months lifestyle interventions, and the results are hard to replicate in independent studies.

The importance of genetic studies in modern sport increases every year. Consequently, it is significant to discuss the achievements, hopes, and fears associated with the rapid development of molecular biology in sport and medicine sciences.

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SECTION III

Genetics of musculoskeletal exercise-related phenotypes

CHAPTER TWELVE

Genetic and epigenetic determinants of muscle mass

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12.1 Inheriting muscle mass

Skeletal muscles occupy 40%–50% of body weight and perform a wide range of functions, such as force production for locomotion, force production for breathing, and heat production (Bottinelli and Reggiani, 2000). The realization that skeletal muscle is an endocrine organ capable of secreting proteins termed "myokines," which participate in tissue crosstalk, provided a critical link in the exercise-health paradigm. There is emerging evidence that myokines may have significant antineoplastic benefits and may represent a major mechanism through which the salutary benefits of exercise are transduced (Whitham and Febbraio, 2016). Muscle mass is an important factor of the physical qualities of the person, is the basis of its athletic achievements, and good health, and an indicator for the duration and quality of life (Trombetti et al., 2016). The number of muscular fibers, their composition, and the level of hypertrophy are those intramuscular factors, which encompass one of the most important physical qualities—the force—the ability to overcome resistance or to counteract it by way of muscular activity.

With age, the loss of muscle mass is accompanied by fatty infiltration and fibrosis of muscle (Doherty, 2003). A wide range of diseases is accompanied by a decrease in muscle mass (Engelen et al., 2000; Elkin et al., 2000; Martin et al., 2013). Loss of muscle mass (sarcopenia) can lead to a loss of motor capacity (Correa-De-Araujo and Hadley, 2014). Among the possible reasons of sarcopenia are disbalance of protein degradation and its synthesis, often caused by chronic inflammation and changes in the concentration of myokines (Han et al., 2018; Wilkinson et al., 2018). Every year the United States spends 18 bln. Dollars on the treatment and prevention of sarcopenia (Janssen et al., 2004). An increase in the number of elderly people with sarcopenia is a heavy burden for society (Beaudart et al., 2014).

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The dimensions of muscle mass depend on the processes of the muscle plasticity, which is reflected by the pronounced adjustments seen in muscular force, endurance, and contractile velocity of mammalian skeletal muscle because of an alteration in demand (Hoppeler, 2016). Some forms of exercise training, such as strength training and resistance training, can produce an enlargement of muscle, known as muscle hypertrophy, which is resulted from an increase of muscle fibers cross-sectional area (CSA). This process includes replication, maintenance, and rearrangement of DNA, through synthesis and RNA processing (transcription) and culminating with the synthesis and processing of regulatory proteins (translation) (Fluck, 2006).

The reverse process, which is observed with age, during immobilization, the reduction of physical activity, is called atrophy. All types of muscle plasticity are controlled by genetic factors, whose influence begins in the early stages of embryogenesis. Skeletal muscle mass (SMM) depends on the interaction of several signal systems (Fernandes et al., 2012). In physiological conditions, the processes of hypertrophy and atrophy are being coordinated by the balance between protein synthesis and proteolysis (Sakuma and Yamaguchi, 2015). The most important factors, which regulate the synthesis and degradation of the proteins of skeletal muscle include phosphatidylinositol-3-Kinase (PI3K), serine/threonine kinase (Akt), mammalian target of rapamycin (mTOR) pathway, SRF-dependent signaling (serum response factor), and the ubiquitin-proteasome system (UPS). According to the recent literature analysis, in mice genes belonging to three signaling pathways can induce hypertrophy: (a) IGF1—Akt-mTOR pathway, (b) myostatin-SMAD signaling, and (c) the angiotensin-bradykinin signaling pathway. Knockout, knockdown, overexpression, or a higher activity of these genes causes overall muscle hypertrophy (Verbrugge et al., 2018). Possibly, genes for proteins that take part in the work of these signal systems may play an important role in determining the degree of hypertrophy of skeletal muscle.

Indices of muscle mass, allowing us to assess it quantitatively, are absolute and of relative weight, lean body mass, and CSA. Indicators of the lean body mass, consisting mainly of a SMM, are important factors of physical strength, endurance, and longevity. For several decades, in the "pre-genomic period," employing the family and twin methods, it has been proven that individual differences in the development of muscle mass, the main component of the fat-free mass (FFM), was determined by significant genetic contribution. Starting with works of Bouchard et al. (1988), Borecki et al. (1991) on relative's body evaluation, it was found that hereditary similarity (familial resemblance) indicated a total transmissible variance of 40%–50% for FFM. In several studies, a degree of inheritance of the FFM appeared to be from 43% (Medina-Gomez et al., 2017) to 52%–60% (Arden and Spector, 1997). Heredity index (H^2) of the SMM, which was determined by dual-energy X-ray absorptiometry technology, totaled 0.809 (81%) (Livshits et al., 2016).

12.1.1 The role of molecular genetic markers in the heredity of muscle mass

As genetic markers are more often a single-nucleotide polymorphism, are less often a copy number variation (CNV), although some studies have claimed that CNVs can explain more parts in genetic differences from SNP (Hurles et al., 2008). A huge number of works has been devoted to searching the possible associations between certain genetic polymorphisms and muscle phenotype. The possible genes in older people were recognized as belonging to the angiotensin-converting enzyme (*ACE*), gene I/D, α-actinin-3 (*ACTN3*) R577X, myostatin (*MSTN*) K153R, and others (Garatachea and Lucía, 2013).

Pathological changes in muscles can also have genetic causes. Thus, the cause of such disease as myotonic dystrophy (DM1), characterized by progressive muscular weakness and atrophy, is an increase in a number of sequences (CTG·CTG) (André et al., 2018).

Nonetheless, in the research conducted in the past few decades, it has been shown that in the case of multifactor signs, the contribution of single genetic marker is impossible to consider. Their information value is only in the sum of sets of these polymorphisms (Boyle et al., 2017). The main scientific trends in recent years have its place in Genome-Wide Association Studies (GWAS). This is a direction of research, engaged in search of communication relationships between genetic markers with the phenotypical signs. In the GWAS directory (MacArthur et al., 2017) upon request "lean body mass" was linked to 13 studies; a request "muscle measurement"—to five studies. The models of transgenic animals also allow us to establish genes, whose structure affects the muscle mass. Methods, which were used in these cases, are knockout/down/in, Zink finger nucleases, Crispr Cas system, etc. (Verbrugge et al., 2018). Even though the high contribution of the genetic factors in the inheritance of the properties of skeletal muscle was proved, and despite the immense number of research on finding the genetic markers of sarcopenia, the relatively slow progress in determining the key factors, prompted the doubts about the possibility of establishing an exact file of genetic factors causing sarcopenia and hypertrophy of muscles (Roth, 2012; Karanikolou et al., 2017). These reservations are strengthened by the fact that the association of many genes with muscle mass detailed in some studies was not repeated in others, and most of the markers associated with the skeletal muscle-trait possess a low contribution to the variation of indicators. Widely used next-generation sequencing (NGS) technology for a genotype of large populations caused also several drawbacks, one of which is impossible to identify rare options that can contribute to the formation of the phenotypes and disease development. As most protein-encoded variations are evolutionary recent, some researchers use genes, but not variations, for calculating gene-specific mutation tolerance score (Roca et al., 2018). Despite these arguments, genetic factors, along with the epigenetic factors, are still considered as the important factors in the development of skeletal muscle, as the informative indicators of forecasting of their status, and the studies of genetic factors have the applied and basic, fundamental importance.

A series of works investigated genes associated with FFM of muscle, in most works discovered by polymorphisms associated with lean mass (LM), did not possess a genome-wide significance level (Guo et al., 2013; Liu et al., 2009). Analysis of full-genomic studies that were searching for the relationship between genetic markers and nonfat weight of whole body [total-body lean mass (TBLM)] and FFM of limbs [appendicular lean mass (ALM)] set up a list of genetic markers presented in Table 12.1.

In one of the first GWA studies, it was determined that an important gene that makes a significant contribution to the LBM variation is *TRHR* (thyrotropin-releasing hormone receptor), which belongs to the G protein-coupled receptor 1 family (Liu et al., 2009). Further, in this study two SNPs (re16892496, rs7832552) were found, and informative value of which has been confirmed also in replicating studies on three populations (Liu et al., 2009). Later in search of the possible associations between the polymorphisms of the gene *TRHR* and FFM in Brazilian older woman, it was found that AFFM (appendicular FFM) and relative AFFM are statistically distinguished in persons with different genotype of rs16892496 (Lunardi et al., 2013), indicating that the *TRHR* gene might be an important candidate for interindividual differences in muscular phenotypes.

The study of SNPs in postmenopausal Japanese women revealed that the rs12409277 (*PRDM16*) is associated with lean body mass (Urano et al., 2014). PRDM16 is a transcript of the coregulator, which takes part in the differentiation of myoblasts. The rs12409277 SNP affects the transcriptional activity of *PRDM16* gene. A replacement of T on the C reduces the ability of this nuclear protein to bind with DNA (Urano et al., 2014).

By researching gene variants of the enzyme methyltetramethicdratase [methylenetetrahydrofolate reductase (*MTHFR*) with variation in LBM and fat body mass (FBM)] it was found that *rs2066470*, *rs4846048*, and *rs3737964* are significantly associated with LBM (Liu et al., 2008). Enzyme MTHFR catalyzes the recovery of 5,10-methyltetrahydrofolate into 5-methylhydrofolate, which is an active form of folic acid, necessary for the formation of S-adenosyl methionine from homocysteine, which plays an important role in the process of DNA methylation. It is well known that DNA methylation controls the activity of genes, including those involved in the process of adaptation to the muscular activity and to hypoxia, and responsible for the growth of muscle tissue and synthesis of mitochondria. Hypomethylation of DNA can lead to the increase of the longitudinal and lumbar myotubes, that is, to the hypertrophy of the muscular tissue (Terruzzi et al., 2011). A reverse dependence between homocysteine level in plasma women's handgrip strength and their physical capacity was confirmed (Swart et al., 2013).

Using GWAS, conducted with the participation of a large number of Chinese individuals, it was found that TBLM is associated with CNV2073 (Hai et al., 2011). Two genes, *GGREM1* (gremlin1) and *CHRFAM7A*, are in the region 15q 13.3, which is

Table 12.1 Characteristic SNP associated with nonfat body mass and appendicular lean mass

Gene	Polymorphism	Allele	Associate	Functional value gene	Populations IA	Source
TNRC6B	rs733381	A/G	WBLM	Encoded a regulators of gene transcription	European	Karasik et al. (2018)
MC4R	Rs10871777	A/G	WBLM	Affects nutritional behavior	European	Karasik et al. (2018)
HSD17B11 17 β-hydroxysteroid dehydrogenase 1	rs9991501	T/C	FFM	Affects the effectiveness of estrogen and androgen	European	Zillikens et al. (2017)
VCAN Versican	rs2287926	A/G	FFM ALM	Encoded a major component of the extracellular matrix	European	Zillikens et al. (2017)
ADAMTSL3 A disintegrin-like and metalloprotease domain with thrombospondin type I motifs-like 3)	rs4842924	T/C	FFM ALM	Encoded a protein of the extracellular matrix	European	Zillikens et al. (2017)
IRS1 The insulin receptor substrate 1	rs2943656	A/G	FFM ALM	have a central role in the insulin-stimulated signal transduction pathway	European	Zillikens et al. (2017)
FTO the fat-mass and obesity-associated	rs9936385	T/C	FFM	is associated with body mass index and body fatness; act as an amino acid sensor, linking circulating AAs to the mammalian target of rapamycin complex 1	European	Zillikens et al. (2017)
ACVR2B activin A receptor, type IIB	rs2276541	A/G	LM	Encoded a protein that inhibits myostatin	Nonhispanic White, Hispanic, European	Klimentidis et al. (2016)
GIMAP1 GTPase of the immunity-associated protein family	cnv1191	CN1, 2, 3, 4	ALM	Important for skeletal muscle cell survival/death in humans	Caucasian	Ran et al. (2014)

Continued

Table 12.1 Characteristic SNP associated with nonfat body mass and appendicular lean mass—cont'd

Gene	Polymorphism	Allele	Associate	Functional value gene	Populations IA	Source
GIMAP1	rs11769150	C/T	ALM	Important for skeletal muscle cell survival/death in humans	Chinese	Ran et al. (2014)
SERHL Serine hydrolase-like protein	cnv2580	CN1, 2, 3, 4	ALM	Important role in normal peroxisome function and skeletal muscle growth in response to mechanical stimuli	Caucasian	Ran et al. (2014)
SERHL	rs139116	T/C	ALM	Important role in normal peroxisome function and skeletal muscle growth in response to mechanical stimuli	Chinese	Ran et al. (2014)
SERHL	rs139120	G/A	ALM	Important role in normal peroxisome function and skeletal muscle growth in response to mechanical stimuli	Chinese	Ran et al. (2014)
GREM1 Gremlin1	cnv2073	CN = 2, 3, and 4,	TLBM	Regulating the proliferation and differentiation of mononuclear cells in human fetal skeletal muscle	Chinese	Hai et al. (2011))
TCF3 Transcription factor 3			TLBM	The regulation of cell growth, differentiation of muscle cells	Caucasian	Ran et al. (2017)
MBD3 Methyl-binding 3			TLBM	Encoded a subunit of the nurd, a multisubunit complex Comprised of HDAC1, directed associated with MyoD	Caucasian	Ran et al. (2014)
UQCR The Ubiquinol-cytochrome c reductase complex			TLBM	To form a part of the mitochondrial respiratory chain. It may also act as a binding factor for the iron-sulfur protein	Caucasian	Ran et al. (2014)
GLYAT Glycine-N-acyltransferase	rs2507838	A/T	ALM	Important in the detoxification of endogenous and xenobiotic acyl-Coa's and in regulating glucose metabolism and energy metabolism	Caucasian	Guo et al. (2013)

GLYAT	rs7116722	T/G	ALM	–	Caucasian	Guo et al. (2013)
GLYAT	rs11826261	A/G	Alm	–	Caucasian	Guo et al. (2013)
TRHR Thyrotropin-releasing hormone receptor	rs16892496	G/T	Tlbm Alm	Receptor for thyrotropin-releasing hormone	US Whites, ChineseBrazilian	Liu et al. (2009) and Lunardi et al. (2013)
TRHR Thyrotropin-releasing hormone receptor	rs7832552	T/C	Tlbm	–	US Whites, Chinese	Liu et al. (2009)
FADS1 Fatty acid desaturase 1	rs174548	C/G	Alm	Component of a lipid metabolic pathway that catalyzes biosynthesis of highly unsaturated fatty acids from precursor essential polyunsaturated fatty acids	Chinese Subjects Caucasian	Han et al. (2012)
FADS1 Fatty acid desaturase 1	rs174549	G/A	ALM	Component of a lipid metabolic pathway	Chinese Subjects Caucasian	Han et al. (2012)
FADS2 Fatty acid desaturase 2	rs174583	C/T	ALM	Component of a lipid metabolic pathway	Chinese subject	Han et al. (2012)
FADS2 Fatty acid desaturase 2	rs174577	A/C	ALM	Component of a lipid metabolic pathway	Chinese subject	Han et al. (2012)
DCHS2 Dachsous Cadherin-related 2	rs7672337	A/G	ALM	Likely functions in cell adhesion	Chinese subject	Han et al. (2012)
PRDM16 Prd1-BF-1-riz1 Homologous domain containing protein 16	rs12409277	T/C	TLBM	An important role in the differentiation of muscle cells	Japanese	Urano et al. (2014)

covered by CNV2073. One of them *GREMLIN1*, has a key role in the regulation of skeletal muscle formation and repair and is a regulator of myogenic progenitor proliferation (Frank et al., 2006; Canalis et al., 2012).

In bivariate GWAS, it was found that the polymorphisms of rs174583 (*FADS2*), rs174577, rs174549, rs174548 (*FADS1*), and rs7672337 (*DCSS2*) were associated with compressive strength and ALM (Han et al., 2012). *FADS1* (fatty acid desaturase 1)—a code for an enzyme involved in fatty acid unsaturation. Multiple polymorphisms at the FADS locus associated with various metabolic phenotypes especially serum lipid compositions and metabolic perturbations (Wang et al., 2016). In another GWAS study, it was shown an association of polymorphisms of this gene with red blood cell fatty acid level (Tintle et al., 2016). In replication research among the Caucasian male subjects, the information value of only two of these polymorphisms (rs174548 and rs174549) was confirmed. Further, it was not confirmed that an association of rs174548 (*FADS2*) with erythrocytes fatty acid composition and activities of D5D and D6D enzymes (Mazoochian et al., 2018) and with risk of type 2 diabetes (T2D).

When analyzing CNV subjects, an association of two CNVs with ALM was established. These CNVs (CNV1119 and CNV2580) found in genes are important for growth and survival of skeletal muscle cells (Ran et al., 2014). It is established that individuals with a smaller number of duplicates in CNV1119 (CN1, CN2) have higher ALM but with a higher number of duplicates (CN3 and CN4) have the lowest ALM. For comparison, in the case of CNV2580 carriers, CN2 and CN3 had higher ALM than CN4. In subsequent studies, this research group has established the significant associations with LM variation of three genes *UQCR*, *TCF3*, and *MBD3* in one single locus 19p13.3 (Ran et al., 2014).

By meta-analysis of 20 GWAS, from 32 preinstalled SNPs, associated with whole body and ALM in the non-Hispanic White, it was shown that two SNPs (Rs2276541 and Rs2284817) of the gene *ACVR2B* (activin A receptor, type IIB) respectively, are associated with differences in the body mass of the surveyed women. *ACVR2B*-receptor gene is used as a negative regulator for the inhibition of skeletal muscle (myostatin). SNP rs2276541 was significantly associated with the LM. The A allele at this SNP was associated with more LM (Klimentidis et al., 2016).

Several researchers consider ALM more accurate indicator, reflecting the state of skeletal muscle, determined as the sum of LM in the arms and legs. With the help of a full-genomic study (GWAS), FFM of the whole body (TBLM) and limbs [appendicular (arms and legs) lean body mass, ALM] in 38,000 people were measured, and it was found that 21 associated with single-nucleotide polymorphisms with a nonfat mass (13 associated with TBLM and 8 with ALM). In a replication study, it was proven that the statistical probability measures five polymorphisms with total nonfat mass and three polymorphisms with FFM of limbs (appendicular lean body mass) (Zillikens et al., 2017).

Skeletal muscle is a powerful stimulating factor for the development of bone tissue, as ALM is correlated with the bone size of the appendicular skeleton and often its indicators are examined simultaneously. The bivariate GWAS is an effective approach to detect pleiotropic genes for complex traits. In bivariate GWAS it was found that 14 SNP had a statistically significant contribution to both the bone size phenotype, and in ALM as well (Guo et al., 2013). However, in replication study carried out with US Caucasians, the association was confirmed only in three of them with the gene *GLYAT* (Guo et al., 2013). This was confirmed that GLYAT encodes the glycine-N-acyltransferase protein, a metabolic enzyme conjugating glycine with acyl-CoA substrates in the mitochondria and is overexpressed in human muscle in a global map of human gene expression (Lukk et al., 2010). Haplotype allele ATA based on these three SNPs was also associated with ALM-HBS and ALM-ABS in both discovery and replication samples.

Use of bivariate GWAS approach is allowed in make up of several different loci that have a pleiotropic effect and influence on both the TBLM and BMD less head region (TBLH-BMD) in four pediatric cohorts. Among them are the following: *WNY4*, *GALNT3*, *MEPE*, *CPED1/WNT16*, *TNFSF11*, *RIN3*, and *PPP6R3/LRP5*. Variants in the *TOM1L2/SREBF1* locus have a stronger association with TBLM and TBLH-BMD (Medina-Gomez et al., 2017).

When researching metabolome, the three substations were found, which explain 11.1% of variations ALM (unidentified substance X12063, urate, and mannose). X12063 was associated with two genomic regions: CYP3AP1 (cytochrome P450, family 3, subfamily A) and protein-coding SLCO1B1 and SLCO1A2 (solute carrier organic anion transporter family) genes. Urate and mannose were associated each with rs737267 (G/T) and rs1260326 (T/C), respectively. There are several metabolites, having a clear pattern of genetic inheritance, which are highly significantly associated with APLM and may provide an inexpensive and readily accessible biomarker of muscle mass (Korostishevsky et al., 2016).

At genome-wide association analyses for whole-body LM, conducted on 29 samples using three different models, six significant single-nucleotide polymorphisms were reconfirmed (*IRS1*, *HSD17B11*, *VCAN*, *ADAMTSL3*, *FTO*, *MC4R*, *TNRC6B*) and one new (*TNRC6B*) was revealed. All markers were divided into those influencing the LM, as well as fat mass (*FTO* and *MC4R*) and only LM (*VCAN* and *ADAMTSL3*). Increasing the number of alleles, associated with LM from the first group of SNPs led to adverse metabolic profile, whereas LM increasing alleles of SNPs from the second group led to metabolic protection (Karasik et al., 2018).

Skeletal muscle of animals and humans differ in morphological and physiological properties and on the distinctiveness of genetic regulation of hypertrophy of muscle fibers. Thanks to a systematic review of data conducted on transgenic animals, it recognized 47 genes with varying degrees of influence on a hypertrophy of skeletal muscle in mice: *Ski*, *Fst*, *Acvr2b*, *Akt1*, *Mstn*, *Klf10*, *Rheb*, *Igf1*, *Pappa*, *Ppard*, *Ikbkb*, *Fstl3*, *Atgr1a*,

Ucn3, *Mcu*, *Junb*, *Ncor1*, *Gprasp1*, *Grb10*, *Mmp9*, *Dgkz*, *Ppargc1a* (*specifically the Ppargc1a4 isoform*), *Smad4*, *Ltbp4*, *Bmpr1a*, *Crtc2*, *Xiap*, *Dgat1*, *Thra*, *Adrb2*, *Asb15*, *Cast*, *Eif2b5*, *Bdkrb2*, *Tpt1*, *Nr3c1*, *Nr4a1*, *Gnas*, *Pld1*, *Crym*, *Camkk1*, *Yap1*, *Inhba*, *Tp53inp2*, *Inhbb*, *Nol3*, *Esr1* (Verbrugge et al., 2018). Nonetheless, since the methods contributing to hypertrophy were different, it is hardly possible to compare the degree of influence of these genes among themselves. Most expressive genes of the skeletal muscle were identified as *Asb15*, *Klf10*, and *Tpt1*.

12.1.2 Cross-sectional area

CSA is an indicator, which affects the maximum force—the principal capability that the athlete can demonstrate during the maximum voluntary muscle contraction. This force depends on the amount and thickness of the fibers and determines the outcome in such sports as weightlifting, athletics, jumping, sprint running, wrestling, sports, and gymnastics. It also has a significant effect on swimming for short distances, rowing, skating, and some sports games.

There are two different genotypes of the CSA: (1) muscle anatomical CSA—the imaginary cross section of the large muscles, or muscle groups; (2) cross-sectional fiber area of individual muscle fibers, fast-twitch, or slow-twitch. The influence of genetic factors on cross-sectional fiber area is less explored. A hypertrophic effect of Pro12Ala polymorphism *PPARG* on muscle fibers was detected (Ahmetov et al., 2008). *PPARG* Ala-allele increases CSA. The list of polymorphisms that can influence fiber-type composition was recently revealed (Ahmetov et al., 2012).

Widely known in exercise genetics *ACTN3* R577X polymorphism that affects the musculoskeletal functions (Del et al., 2019), is also making a significant contribution to the muscle anatomical CSA. In the study of nontrained Japanese women, it was found that there are no differences in thigh muscle CSA among *ACTN3* R577X genotypes. But in the older group XX genotype a smaller thigh muscle CSA observed, compared to RR and RX genotypes (Maeda et al., 2011)

Animal studies demonstrated that in order to increase a cross section of muscle, it is important to have increased activation of genes *Ski* (ski Oncogene), *Akt1* (Protein kinase B), *Igf1* (insulin-like growth factor 1, and suppression or exclusion of functions of the following genes: *Klf10* (TGFb—inducible early growth response protein 1, Krüppel-like factor 10), *Atgr1a* (Type-1 Angiotensin Ii receptor), and the *Mstn* (myostatin) (Verbrugge et al., 2018). *Klf10* encodes a transcriptional regulator that mediates the effects of TGF-β signaling. Its involvement in the work of skeletal muscle is supported by the fact that the loss of *Klf10* increases fibrosis. Collagen Type I (*Col1a1*) and fibronectin gene expression and protein deposition were increased in skeletal muscle of Klf$10^{-/-}$ mice, contributing to increased fibrosis. Dystrophic *Klf10*-null mice also had reduced grip strength. *Klf10* moderates the fibrotic effects of TGF-β signaling in chronically damaged regenerating muscle (DiMario, 2018).

12.2 The impact of myofibril's structural proteins on a mass of skeletal muscle

One of the particularly important myofibril's structural proteins, which occupies a half of muscle, sarcomere-titin performs a wide range of functions in striated muscle (Hidalgo and Granzier, 2013). Titin adjusts the length of thick filament: it was shown that thick filament length in cardiac and skeletal muscles of a mouse with two deleted titin's C-zone (delete-super-repeats) were reduced (Tonino et al., 2017). Titin regulates passive muscle stiffness and regulates the volume of skeletal mass. In response to exercise-induced tissue damage dislocation and fragmentation, titin mediates adaptive hypertrophic signaling (Krüger and Kötter, 2016). Gene titin (*TTN*) has a high mutation tolerance, so it has a low frequency of rare options (Roca et al., 2018). The polymorphism of this gene associated with a wide range of phenotypic signs and with cardiac and skeletal muscle diseases (Chauveau et al., 2014; Savarese et al., 2018). The truncating *TTN* mutations are a cause of centronuclear myopathies (CNM) (Fattori et al., 2015) and muscle disorders such as titinopathy (Savarese et al., 2018). The different TTN mutations cause truncated proteins resulting in titin molecules that are devoid of the functions performed by titin's M-band segment, including structural and mechano-sensing roles (Ceyhan-Birsoy et al., 2013).

12.2.1 Growth factors

Muscle growth depends on many growth factors. The most important among them is myostatin and GDF11 (growth differentiation factor 11). Myostatin is a well-known control factor of SMM, which affects both the number of myofibril during development and postnatal muscle growth. The loss of the gene of this protein leads to twofold increase in muscle mass (Schuelke et al., 2004). Mutations in these genes have long been studied since they are crucial for muscle activity. Polymorphism of the myostatin gene is indicators of physical capacity, including an ability to produce "peak" power during muscle contractions, as assessed with vertical jump test (Santiago et al., 2011), and with strength training-induced muscle hyperthrophy (Li et al., 2014). K153R polymorphism is associated with obesity, lower muscle strength, and extension of lifespan and with increasing the rate of proteolysis of promyostatin (Szláma et al., 2015).

Because the increase in muscle mass is one of the therapeutic strategies for skeletal muscle diseases, there is a significant rise of research of the actions of various myostatin inhibitors: delivery of neutralizing antibodies against myostatin, deacetylase inhibitors, and follistatin (trichostatin). Most antimyostatin medications are blocking interactions between mature myostatin, the receptor for antibodies, ligand traps, or overexpression of natural inhibitors such as follistatin. Thus, in particular, it is established that a single postnatal intramuscular injection of adeno-associated virus (AAV), which is responsible for coding myostatin-inhibiting proteins [growth and differentiation factor-associated

serum protein1 (GASP-1)], follistatin-344 (FS), follistatin-related gene (FLRG) in normal mice (wild-type), and in mice with muscular dystrophy (Duchenne muscular dystrophy), results in a long-term increase in the size and strength of muscles. The greatest gain in muscle mass was registered in FS-treated animals (Haidet et al., 2008). Chronic treatment of mice with REGN1033 (human monoclonal antibody) increases muscle fiber size, muscle mass, and force production (Latres et al., 2015). New alternative therapeutic approaches, which are based on the use of monoclonal antibodies, that have selective-linking myostatin and GDF11 by blocking their extracellular activation, lead to persistent muscular growth and an improvement of physical capacity in healthy mice (Pirruccello-Straub et al., 2018).

The transcription factors which influence the formation and differentiation of skeletal myoses both on embryonic and in the postnatal level belong to myogenic regulatory factors (MRFs): MyoD, Myf5, myogenin, myogenic regulatory factor4 (MRF4), and myf6 (herculin) (Hernández-Hernández et al., 2017). In adult individuals, satellite cells are activated only when the muscles are damaged and expressed for MyoD, Myf5. The data exist on the inclusion of myf6 in the processes of muscular hypertrophy and the changing ratio of types of muscle fibers in the process of resistance training. The positive correlation was established between the muscle fiber CSA and mRNA expression of MyoD ($r = 0.85$, $P = .0001$), myogenin ($r = 0.87$, $P = .0001$), and IGF-I ($r = 0.88$, $P = .0001$) (Aguiar et al., 2013).

A loss of MRF4 in the skeletal muscle of adult persons leads to muscle hypertrophy and counteracts the development of degenerative atrophy, which is mediated by MEF2 (Schiaffino et al., 2018). It was shown that gene polymorphisms of MRFs mediate the effect on the properties of skeletal muscles in chickens and other livestock animals (Yang et al., 2015) and these effects may be used in livestock breeding. It established an association of C964T gene polymorphism MYF6 in the nontranslated area of mRNA (rs3121) with the cross section (PPP) of muscle fibers. It was recognized that T/T genotype is favorable for the development and manifestation of the qualities of endurance, and that gene polymorphism MYF6 can be associated with human physical activity (Druzhevskaya et al., 2008).

12.2.2 Noncoding RNA

Some of the key factors in the regulation of muscular development, maintaining homeostasis and metabolism, are noncoding RNA (including micro-and long noncoding RNA). Even though their biological role began to study not so long ago, the importance of their participation in a wide range of biological processes is already certain. Deviation of the expression of noncoding RNA from the norm is associated with various muscular diseases, such as muscular dystrophy, cachexia, and sarcopenia (Nie et al., 2015). Based on size, noncoding RNA is traditionally divided into two large classes: small noncoding

RNA (miRNA) and long noncoding RNA (lncRNA). Participation of miRNA and lncRNA in the process of regeneration of skeletal muscles after the damage was recently revealed (Gonçalves and Armand, 2017). Especially, their participation in metabolic processes in skeletal muscles and myogenesis is important (Hagan et al., 2018).

It was also shown that miR-487b-3p overexpression significantly suppressed C2C12 myoblast proliferation and differentiation, whereas the inhibition of MiR-487b-3p accelerated C2C12 myoblast and differentiation (Wang et al., 2018).

Changes in miRNA are important in atrophy associated with congenital myopathies, maintaining fiber type, and muscle adaptation to exercise (van Rooij et al., 2009; Nielsen et al., 2010). MiRNAs exported into the circulation are also potential biomarkers for specific disease phenotypes and for following disease progression (Etheridge et al., 2011).

Increased expression of miR-675/H19 and altered methylation of the H19 imprinting control region are associated with a low FFMI in patients with COPD but not in a healthy community dwelling of older men, suggesting that epigenetic control of this loci may contribute to susceptibility to a low FFMI (Lewis et al., 2016).

It was shown that 23 miRNAs (including let-7a-5p, 95, 148a-3p, 376a-3p) were differentially regulated after acute resistant exercise and 26 miRNAs, especially 30d-5p and 376a-3p, and also—after 12 week of lower body RE (resistant exercise) training, in the skeletal muscle between high and low responders, indicating that the expression patterns of several miRNAs are altered by acute or chronic RE, and that miRNAs are involved in skeletal muscle adaptation to RE training (Ogasawara et al., 2016).

Physical training aimed at the development of endurance, regulate the level of muscles lncRNA PINK1 antisense RNA and so impact the processes of splicing PINK1 (PTEN-induced putative kinase 1), a metabolic gene, associated with Parkinson's disease (Scheele et al., 2007).

It was found that the gene LncMyoD is able to control the proliferation of myoblasts and influence the regeneration of muscles after damage (Gong et al., 2015). Knockdown of LncMyod prevents the myogenesis, suppressing the expression of genes in mature muscle cells. More than 1000 interethnic lncRNA in the muscular cells of the line C2C12 takes part in the formation of muscle fibers at the level of myotubes. In the enhancer region of the gene MyoD, it identified the two lncRNA: ^{DRR}RNA (distal regulatory region) and ^{CE}RNA (core enhancer, chief enhancer). It is believed that ^{CE}RNA (cis-) facilitates chromatin availability and stimulates gene expression, and ^{DRR}RNA functions in tranc- and leads to increased expression of myogenin, key myogenic transcription factor (Mousavi et al., 2013).

A new group of lncRNA was recently identified—lnc-mg (myogenesis-associated lncRNA), which takes part in the regulation of muscle cell differentiation and skeletal muscle development (Zhu et al., 2017). Conditional knockout of ln-mg leads to muscle atrophy and decreases of muscular endurance. Contending to MiRNA-125b, ceRNA, is able to modulate myogenesis, by controlling the level of insulin-like growth factor 2 and

thus—promotes myogenesis. Further, in lnc-mg transgenic mice, an increase in CSA of muscle fibers was observed. It was observed that LncRNA muscle anabolic regulator 1 (MAR1) was highly expressed in mice skeletal muscle and was positively correlated with muscle differentiation and growth in vitro and in vivo. It is of interest that MAR1 acts as a miR-487b sponge to regulate Wnt5a protein, an important regulator during myogenesis (Zhang et al., 2018).

Possibly, given the key role of noncoding RNA in myogenesis and regeneration, the influence on satellite cells, polymorphisms of these genes will be able to affect the growth and development of skeletal muscles. For example, in the chicken muscles, it was established that two sequence variants (g. 1198A > G and g. 1238-1239del/insGA), located in exon 1 of pouMU1, are associated with significant differences in muscle development and growth (Ren et al., 2017).

12.3 Genetically based changes of muscle mass under influence of physical activity

There is a vast number of studies that have established muscle growth under the influence of strength training (Thomas, 2011; Gomes et al., 2018). Resistance training and combined aerobic and resistance training have been proven to maintain muscle performance of people of different age (Ferrari et al., 2016; Liberman et al., 2017).

The differences in the increase of whole-body mass and CSA after resistance exercise training allow us to differentiate between the low and high skeletal muscle hypertrophic responders (Roberts et al., 2018). However, the key factors in the formation of such a phenotype are not clear: possibly, high levels of IFG-1, satellite cell counts, degree of ribosome biogenesis, microRNA, connective tissue properties, as well as "favorable" genetic variation play a role. At present, the combination of different SNPs/insertions-deletions/tandem repeats is likely prevalent in high versus low skeletal muscle hypertrophic responders.

Attempts to create a predictor of skeletal muscle development method, despite existing difficulties are in progress. With the aim to assess the predictive power of data-driven GPSs on baseline muscular phenotypes and muscle adaptations during exercise in a healthy elderly population whose whole-body SMM and isometric knee extension strength (PTIM60) were measured before and after 1-year intervention program of the exercise (FIT and WBV). The analyses of results show that four SNPs (*ACVR1b*; rs2854464; *FST*: rs3797297; *IGFBP3*: rs3110697; and *TTN:* rs10497520) were significantly related to baseline PTIM60. Data-driven GPS could explain 3.2% of the variance in isometric knee extensor (He et al., 2018). Six SNPs (*CCL2*: rs4586; *CCR2*: rs768539; *GR/NR3C1*: rs6190; *METTL21C*: rs2390760; *MSTN*: rs2390760; *SPP1*: rs10516796) were found significantly related to SMM changes in the exercise groups. Eight SNPs (*AKT1:* rs1130214; *DNMT3L*: rs7354779; *IGFm BP3*: rs3110697; *IL15RA*:

rs2228059; *MSTN*: rs1805086; *MTRR*: rs162040, rs7703033; and *SPP1*: rs10516796) were found to be significantly associated with the change in knee extensor strength by training. The GPS explained part of the interindividual variance of training response with some DNA methylation-related genes involved in the adaptive process.

Despite the critics of the perceived small value of genetic markers (Pickering and Kiely, 2017), the search of genetic-based algorithm for personalized resistance training and for prognosis of physical qualities of skeletal muscles, endures. Such an algorithm of 15 polymorphisms led to the conclusion that resistance training is more effective (Jones et al., 2016).

12.4 Epigenetic factors influencing the muscle mass

The development of epigenetics studies has allowed us to realize that processes of muscular hypertrophy, and therefore, the size of muscle mass can be programmed by changing the methylation of the DNA, in genes associated with muscle growth and their differentiation (Howlett and McGee, 2016). It was shown that DNA methylation decreased with exercise (Brown, 2015). It was established that aerobic exercise in mice altered methylation of 2762 genes (3692 CpG sites) in their putative promoter regions (Kanzleiter et al., 2015). The comparison with the level of expression allowed us to distinguish 200 genes with a negative correlation between methylation and changes in expression in response to physical exertion: in 66 hypomethylated allergenic genes was observed the growth of expression and in 134 hypermethylated—the decrease of expression. Most of these genes have been linked to muscular growth processes and their differentiation, the lesser part—with the regulation of metabolism. Among the list of genes are those genes that regulate the expression of myogenic regulatory factors (PlexinA2) and taking part in the development of muscular hypertrophy (Igfbp4), as well as innervation of motor neurons. Increased methylation at the same time was observed on the binding sites of myogenic regulatory factors MyoD and myogenin.

In another study in search of associations between DNA methylation and SMM in 50 discordant MZ twins, it was found that 36,081 signals from which in the replicative studies on 1196 persons just 134 was confirmed (Livshits et al., 2016). Seven associations between methylation and SMM replicated at a false discovery rate of less than 0.1 were located in or near genes *DNAH12*, *CAND1*, *CYP4F29P*, and *ZFP64*.

Genome-wide DNA methylation investigations and gene expression analysis of human skeletal muscle showed that resistance exercise-induced muscle hypertrophy is followed by epigenetic modification of 17,565 CpG sites. A total of 9153 sites were hypomethylated and 8212-hypermethylated (Seaborne et al., 2018). During the exposure to 7-weeks unloading the frequency of hypomethylated sites did not change, but after reloading—surged to 18,816, while the frequency of hypermethylated cites did not change. It was shown that *AXIN1*, *GRIK2*, *CAMK4*, and *TRAF1* as

hypomethylated genes with enhanced expression after loading that maintained their hypomethylated status even during unloading where muscle mass returned to control levels, indicating a memory of these genes' methylation signatures following earlier hypertrophy.

12.5 Conclusion

The current review provides evidence that the genetic and epigenetic factors determine the development of the skeletal muscle tissue and its characteristics. Polymorphism of genes of structural proteins within sarcomeres, myogenic regulatory factors, and genes of the proteins belong to the list of molecular-genetic markers, associated with fat-free mass and hypertrophy. Review of the current literature has established 28 markers associated with lean body mass. An impact of physical exercise on skeletal muscle and the development of skeletal muscles are indirectly affected by epigenetic mechanisms and by the action of noncoding RNA (miRNA and lncRNA), which are also participating in the mechanisms of muscle memory.

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Further reading

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CHAPTER THIRTEEN

Genetics of flexibility

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13.1 Preliminaries

Flexibility is a complex phenotypic trait and represents an important physical fitness component in many sports and performing artists (Simmonds, 2010; Donti et al., 2016; Silva et al., 2019; McCormack et al., 2019). An excess of flexibility, as joint hypermobility (JH), is a desirable feature in certain sports and it is a highly heritable condition in which joints have a range of motion (ROM) beyond normal limits (Gannon and Bird, 1999; Simpson, 2006).

The cut point at which flexibility becomes JH is somewhat arbitrary even when standard scales not applied to athletes are used (McCormack et al., 2019). Different studies showed that although this high level of flexibility is a desirable characteristic in sports where ROM is especially important for success (Bulbena and Pailhez, 2011), JH is reportedly an intrinsic risk factor for injuries, including tendon and ligament injuries (Knapik et al., 1992).

Although the level of flexibility is believed to be related to the risk of muscular injury and athletic performance, no consensus has currently been reached on the importance of flexibility in sports where a high ROM is not required for success.

The level of flexibility varies among individuals and it is influenced by genetic factors (heritability estimates = 50%) (Chatterjee and Das, 1995). Inherited characteristics include both the shape of the articulating surfaces of bone, muscle, and collagen structure (Beighton et al., 2011; Foley and Bird, 2013). In support of this, studies report a familial predisposition to greater flexibility or JH pathologies (i.e., shoulder dislocation) (Beighton and Horan, 1970; Bridges et al., 1992; Dowdy and O'Driscoll, 1994) and a greater prevalence of JH in female identical twins compared with fraternal twins (Hakim et al., 2004).

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Flexibility and JH maybe also partly acquired, in the sense that the ROM can be increased, with specific training (Grahame, 2003).

To date, most of the genomics studies on flexibility have been focused on polymorphisms in muscles, ligaments, and other tissues associated with ROMs and muscle stiffness, while the genomics studies on JH in extreme phenotype have analyzed the genetic mutations within genes encoding for structural components of the extracellular matrix (ECM) or ECM regulating components. Currently, only one genome-wide association study (GWAS) has been carried out on JH identifying 18 loci associated with generalized joint laxity (Pickrell et al., 2016).

The present chapter provides a background on the studies currently performed on the field of genetics of flexibility and JH in extreme phenotype. Starting with a detailed description of the phenotype of interest, this chapter illustrates the genetic variants candidate to influence the ROMs and muscle stiffness and the genetic mutation associated with symptomatic JH.

13.2 Phenotype definition

Flexibility is one of the most important components of physical fitness, along with cardiorespiratory endurance, muscle strength, etc. Although flexibility is believed to be related to the risk of muscular injury and athletic performance, no consensus has been reached on the importance of flexibility in sports in which a very good ROM is not essential for the success. This can be explained, at least in part, by the lack of consensual definitions and measures of flexibility, and a general lack of scientific understanding of the determinants of flexibility. This section describes fundamental concepts about flexibility and its measurement in human experiments, with an emphasis on "muscle stiffness," which is the primary determinant of flexibility.

13.2.1 Definition of flexibility

There seems to be little agreement on the definition of the so-called "normal" flexibility. In the fields of sports medicine, sports science, and health science, one of the simplest definitions of flexibility is the maximum ROM of a joint or a series of joints. Flexibility is also defined as the capacity of a joint to move fluidly through its full ROM (Heyward, 1984), the total achievable excursion (within limits of pain) of a body part through its potential ROM (Saal, 1998), the ability of a person to move a part or parts of the body in a wide purposeful movements at the required speed (Galley and Forster, 1987), and the ability to move a joint through a normal ROM without undue stress to the musculotendinous unit (Chandler et al., 1990). Taken together, flexibility can be defined as the ability to move a part or parts of the body over a wide range, with small resistance.

Flexibility can be classified broadly into two types: static flexibility and dynamic flexibility. Static flexibility is defined as the ROM in a joint or a series of joint, with no

emphasis on speed. Typically, measurements of static flexibility are performed when the individual is instructed to relax. Among the several methods to evaluate static flexibility, the classic tests are the toe-touch and the sit-and-reach (although, strictly speaking, these tests are performed under active conditions, i.e., during contracting trunk muscles). Another classic test is the unilateral passive straight-leg-raise. Dynamic flexibility refers to the ability to move a joint or a series of joints in the performance of physical activity at either a normal or rapid speed (Corbin and Noble, 1980). Another term similar to dynamic flexibility is ballistic flexibility. Tests of ballistic flexibility usually involve bouncing and rhythmic motion, whereas dynamic flexibility tests do not necessarily require ballistic or fast types of movements. The nature of dynamic flexibility is complex because several factors like muscle strength and coordination, as well as the state of static flexibility, are involved. Thus, careful attention is necessary when interpreting the results of dynamic flexibility tests.

JH and joint laxity are terms that are related to flexibility, but they are not synonymous. Flexibility refers to the extensibility of particular tissues to allow normal or physiologic motion of a joint or a series of joints, while joint laxity refers to the stability of a joint and usually represents a function of the joint capsule and ligaments. Excessive joint laxity can be due to a chronic injury or a congenital or hereditary condition. In other words, the term flexibility is used to express the degree of normal motion whereas joint laxity is used to express the degree of abnormal motion of a given joint. The term JH is used to express the motion in excess of the accepted normal ROM in most joints. JH is seen frequently in healthy individuals who do not have complaints (Simpson, 2006). The prevalence of JH varies from 10% to 15%, with important differences among race, sex, and age, and it is three times more frequent in women than in men (Beighton et al., 1973, 1989). JH is not a medical problem and it may even be an advantage in sports that required a high ROM for success, for example, artistic and rhythmic gymnastics, karate, figure skating, etc. As shall be illustrated later in the chapter, JH becomes a medical problem when it is deemed to be responsible for emerging symptoms, of which pain and instability are the most important (Grahame, 2010).

13.2.2 Flexibility measurements

Goniometry is a representative measure of the ROM of a joint or a series of joints, regardless of the type of flexibility. ROM can be quantified in two ways: in linear units (e.g., centimeters and inches) and in angular units (degrees of an arc). ROM in the toe-touch and sit-and-reach tests is expressed in centimeters whereas ROM in the straight-leg-raise test is reported in degrees.

In the fields of sports and rehabilitation, measures of flexibility are usually performed to evaluate the ability of a skeletal muscle to lengthen. The aforementioned flexibility tests (i.e., toe-touch, sit-and-reach, and the straight-leg-raise) are reproducible and are

considered to evaluate the hip flexors (specifically the hamstring) extensibility. However, there are some concerns about these tests. Firstly, the endpoint of the straight-leg-raise test (i.e., hip flexion angle) is generally determined based on (i) the tightness or firm resistance felt by the examiner or (ii) the pain in the hamstring reported by the examinee. Consequently, the straight-leg-raise test can be strongly influenced by the examiner's or examinee's subjectivity (including individual variability in muscle stretch tolerance) (Weppler and Magnusson, 2010; Ayala et al., 2012). Additionally, although several variations of the sit-and-reach test have been proposed (e.g., classic sit-and-reach test, modified sit-and-reach test), they are influenced by factors other than hamstring extensibility, such as spinal and shoulder flexibility as well as muscle stretch tolerance (Mayorga-Vega et al., 2014; Muyor et al., 2014). In other words, the toe-touch, sit-and-reach, and the straight-leg-raise tests are multifactorial. Thus, in order to properly evaluate flexibility, objective (i.e., without the sensation of examiners and examinees) approaches which are not influenced by anthropometric factors such as spinal and shoulder flexibility are required.

As previously mentioned, flexibility (especially static flexibility) can be defined as the ability to widely move a joint or a series of joints with small resistance, that is, the ease of movement within the obtainable ROM. Thus, in an experimental laboratory setting, static flexibility in a joint is evaluated by measuring passive resistance (i.e., torque) as well as joint angle. The slope of the torque-angle curve is assessed as the joint stiffness; stiffness is a term used to describe the load (force) required to achieve a certain deformation of a material, and the inverse of stiffness is compliance. Some studies have shown significant (but moderate) relationships between joint stiffness and maximum joint ROM (Magnusson et al., 1997; McHugh et al., 1998). Hence, joint stiffness assessed from the torque-angle curve is considered as an indicator of muscle stiffness, and accordingly, it has been suggested that individuals with greater joint ROM have more compliant muscles (Magnusson et al., 1997). However, it should be noted that passive torque at a joint is not a measure specific to the responses of the muscles involved. Instead, it is related to the mechanical (viscoelastic) responses of the entire musculo-articular complex including both muscular and nonmuscular tissues such as tendons, ligaments, and joint capsules. In other words, joint stiffness assessed from the torque-angle curve does not allow precise and direct assessment of muscle stiffness. Recently, elastographic techniques have been used for in vivo noninvasive assessment of the stiffness of biological tissues, by measuring shear wave propagation speed. In particular, ultrasound-based shear wave elastography is a relatively new imaging technique which facilitates quantification of the shear modulus (a measure of stiffness, expressed in kPa) of a localized area of tissue, independently of the influences of other anatomical structures (Bercoff et al., 2004; Gennisson et al., 2013). The results of an animal experiment have demonstrated that the muscle shear modulus measured using ultrasound shear wave elastography is highly correlated with Young's modulus (a fundamental material property) of the muscle, measured using a traditional

stress-strain test ($r \geq 0.957$) (Eby et al., 2013). An increasing number of studies using ultrasound shear wave elastography have quantified the stiffness of individual muscles of muscle groups such as the hamstring (Miyamoto et al., 2017, 2018b), quadriceps femoris (Xu et al., 2018), and triceps surae (Hirata et al., 2016; Le Sant et al., 2017).

Recently, we revealed that the ROM in the straight-leg-raise and sit-and-reach tests is significantly, but weakly-to-moderately, correlated with the muscle shear modulus of each of the hamstring (i.e., biceps femoris, semitendinosus, and semimembranosus) ($r \leq 0.484$) (Miyamoto et al., 2018a). This finding suggests that the straight-leg-raise and the sit-and-reach tests are strongly influenced by factors other than the hamstring muscle stiffness and therefore might not accurately evaluate hamstring stiffness. However, it should be noted that ultrasound shear wave elastography can provide information on the stiffness of only a limited region of a muscle of interest since the shear wave propagation speed is determined from a relatively small region of tissue due to the limited size of the ultrasound probe.

13.2.3 Influencing factors for ROM and stiffness

The ROM in a joint or a series of joints is influenced by the endpoints of the examiner's or examinee's sensation (sensory perception) as well as the mechanical (viscoelastic) responses of the entire musculo-articular complex. Although the sensation of the examiner or examinee is the most frequently used endpoint when determining the ROM in human flexibility research, there is little consensus regarding which sensation is most relevant to muscle stiffness among the typically wide range of sensations, such as resistance, discomfort, and pain. Even though joint flexibility is evaluated by measuring both torque and joint angle, the joint stiffness calculated at the end-ROM which differs between individuals, is influenced by the effect of sensation. Thus, it is desirable to calculate joint stiffness at a certain joint angle common to all examinees and, if possible, for the muscle group stretched to a tensioned state without pain.

In general, flexibility may be considered a common (i.e., more or less uniform) characteristic throughout the body. In fact, however, there is evidence that flexibility does not exist as a general characteristic but is specific to a particular joint and joint action (Corbin and Noble, 1980). For example, sufficient ROM in the shoulder joint does not indicate sufficient ROM in the hip joint. Similarly, adequate ROM in one hip joint does not necessarily ensure adequate ROM in the other hip joint. In other words, the measurement of ROM in one joint cannot be considered as a valid alternative or predictor of ROM in other body parts. Although these differences might be due to the specialized mechanical strains that the individual has imposed on his or her connective tissues in a particular joint, the precise mechanism remains unclear.

As one of the determining factors for joint flexibility, one may expect that tendon extensibility or stiffness is involved. However, tendon elongation during passive

lengthening (stretching) is smaller than during maximal voluntary contraction. Furthermore, given that muscle and tendon are placed in series, the stiffness of a muscle-tendon unit (MTU) is expressed as

$$\frac{1}{S_{\mathrm{MTU}}} = \frac{1}{S_{\mathrm{muscle}}} + \frac{1}{S_{\mathrm{tendon}}}$$

where S represents stiffness. Tendon stiffness is much higher than muscle stiffness (Stolov and Weilepp, 1966). Thus, the MTU stiffness depends considerably on the muscle stiffness. Taking these into consideration, tendon tissues may not substantially influence joint flexibility including ROM and joint stiffness. On the other hand, from a mechanical point of view, it is reasonable to consider that among the factors other than sensation, the stiffest muscle is the primary determining factor for the ROM and joint stiffness.

Studies with animals have demonstrated that type I muscle fibers exhibit higher stiffness compared with type II fibers (Kovanen et al., 1984; Mutungi and Ranatunga, 1996). This is probably due to differences in titin isoform within each fiber (Wang et al., 1991) and/or in the content of collagen, which is the primary constituent in intramuscular connective tissues such as epimysium, perimysium, and endomysium (Purslow, 1989). Among them, the perimysium is considered a primary extracellular contributor to muscle stiffness (Purslow, 1989; Gajdosik, 2001).

All these factors should be carefully taken into account when genotype:phenotype association studies are performed in the field of flexibility.

13.3 Genetic markers associated with ROMs

Flexibility and JH varying among individuals and are a highly hereditable traits influenced by genetic factors (heritability estimates = 50%) (Chatterjee and Das, 1995; Hakim et al., 2004). In support of this, Hakim et al. (2004) analyzed 483 monozygotic and 472 dizygotic female twin pairs and showed a strong genetic component to self-reported JH (Hakim et al., 2004). Moreover, a classic twin study on 300 monozygotic and dizygotic male twins showed that the proportion of variance in lumbar ROM attributable to genetic influences (heritability estimate) was 47% (Battiè et al., 2007). The extent of lumbar ROM in flexion was predominantly determined by genetic influences (64%), while the extension was influenced to a somewhat greater degree by environmental and behavioral factors (Battiè et al., 2007). Furthermore, three more studies that analyzed monozygotic and dizygotic twins, children, and young adults showed that 18%–55% of the variation in flexibility (as measured by the sit-and-reach test) could be explained by genetic factors (Chatterjee and Das, 1995; Maes et al., 1996; Okuda et al., 2005). Taken together, the existing studies confirm a role for genetic influences on the individual differences in flexibility. So far, only five genetic markers associated with flexibility (ROMs) have been identified (Table 13.1) and replication

Table 13.1 Gene variants (genetic markers) for flexibility.

Gene	Polymorphism	Allele/ genotype	Subject	Associated phenotype	Reference
COL1A1	rs1800012 T/G	TG + TT vs GG	Males and females	Increase GR	Bell et al. (2012)
COL5A1	rs12722 C/T	CC vs CT	Males and females	Decrease GR, GJL	Bell et al. (2012)
		CT vs TT	Females	Increase GR, JLS	Bell et al. (2012)
		CT vs CC vs TT	Males and females	Increase SLR, SR	Collins et al. (2009)
		CC vs CT vs TT	Males and females	Increase SRL	Lim et al. (2015)
		CC vs TT	Males and females	Lower age-decline in sit-and-reach ROM	Brown et al. (2011)
		CC vs TT	Old individuals (males and females)	Increase sit-and-reach ROM	Brown et al. (2011)
		CT vs CC vs TT	Elite female rhythmic gymnasts	Increase GR	Tringali et al. (2014)
		CT vs CC	Elite female rhythmic gymnasts	Increase GJL	Tringali et al. (2014)
COL12A1	rs240736 C/T	CCvs CT vs TT	Males	Increase GJL	Bell et al. (2012)
	rs970547 A/G	AA vs GA + GG	Females	Increase AKL	Bell et al. (2012)
ACTN3	rs1815739 R/X	XX vs RX vs RR	Females	Decrease sit-and-reach ROM	Kim et al. (2014)
		RR vs RX vs XX	Females	Decrease sit-and-reach ROM	Zempo et al. (2016)
		RR vs RX vs XX	Males and females	Decrease sit-and-reach ROM	Kikuchi et al. (2017)
		RR vs RX vs XX	Males	Decrease ROM elbow-flexion	Kikuchi et al. (2018)
ESR1	rs2234693	CC vs CT vs TT	Males and Females young adults	Decrease muscle stiffness	Kumagai et al. (2019)
	rs9340799	AA vs AG vs GG	Males and Females young adults	Increase muscle stiffness	Kumagai et al. (2019)

GR, Genu recurvatium; *GJL*, general Join Laxity; *SRL*, passive straight leg raise; *WBJL*, whole body join laxity; *JLS*, join laxity score; *AKL*, anterior knee laxity.
The underlined genotypes are associated with flexibility phenotype.

studies are needed to verify those. This section provides an overview of the most studied genetic markers associated with ROMs.

13.3.1 ROMs and the COL5A1 rs12722 polymorphism

Since collagen, type V, alpha-1 gene (*COL5A1)* mutations cause classic Ehlers-Danlos syndrome (EDS), a condition described later in this chapter and characterized by JH and other connective tissue-related symptoms (Malfait et al., 2010), the association between *COL5A1′* untranslated region (rs12722) and ROM has been investigated (Brown et al., 2011; Douda et al., 2008) and it represents today the most extensively studied relationship between genotype:phenotype in the field of genetics of flexibility.

COL5A1 encodes the α1 (V) chain of type V collagen (Wenstrup et al., 2006), a structural protein of the ECM, which plays a crucial role in the regulation of the size and configuration of other abundant fibrillar collagens supporting tendons, ligaments, and muscles (Wenstrup et al., 2011). It has been demonstrated that a common C to T single nucleotide polymorphism within the rs12722 polymorphism may alter *COL5A1* mRNA stability and thereby reduce the production of type V collagen (Laguette et al., 2011). In details, it was hypothesized that individuals with the *COL5A1* TT genotype would have increased type V collagen production (Wenstrup et al., 2006) and thus have less extensible tendon structures, resulting in a lower ROM (Collins and Posthumus, 2011) and in a higher risk to develop soft-tissue injuries (Posthumus et al., 2009; Massidda et al., 2015; Heffernan et al., 2017; Pabalan et al., 2018).

The first study that reported an association between the *COL5A1* rs12722 (T to C) polymorphism and musculotendinous ROMs was performed by Collins et al. (2009). The authors found that the lower limbs of the subjects with CT genotype were less flexible than homozygous subjects (TT and CC genotypes), concluding that the *COL5A1* rs12722 was independently associated with the ROMs measurements tested. In addition, a follow-up study on a larger cohort of apparently healthy physically active Caucasian reported that the *COL5A1* rs12722 CC genotype protected the participants against an age-related decline in sit-and-reach ROM (Brown et al., 2011). When dichotomized in accordance with age, the COL5A1 CC genotype was significantly associated with an increased sit-and-reach ROM in older, but not younger individuals. Similarly, a recent study reported that the *COL5A1*rs12722 polymorphism was not associated with sit-and-reach ROM measurements among young Brazilians (Bertuzzi et al., 2014). Although sit-and-reach ROM was not quantified, the *COL5A1* CC genotype was found to be associated with increased straight-leg-raise ROM in young Korean and Japanese individuals (Lim et al., 2015). Moreover, this study reports also a tendency for the CC genotype to be associated with increased whole-body joint laxity. The *COL5A1* rs12722 CT genotype is also reportedly associated with high joint mobility, the occurrence of genu recurvatum (knee hyperextension), and a higher incidence of injuries in a cohort of

Italian high-level international rhythmic gymnasts, further implicating the involvement of this variant in the modulation of joint mobility (Tringali et al., 2014). Furthermore, Bell et al. (2012) investigated the association among the *COL5A1* rs12722 polymorphism, general joint laxity, ROM and the frequency of ACL injury. They found a significant association in females for both genu recurvatum ($P = .02$) and general joint laxity ($P = .02$). Females with the CT genotype exhibited greater genu recurvatum ($4.6 \pm 4.3°$) than those with either the CC or TT genotype (respectively, $1.7 \pm 2.3°$, $P = .01$; and $1.7 \pm 2.8°$, $P = .02$) and had a greater general joint laxity score (2.4 ± 1.8) than those with the CC genotype (0.9 ± 1.3; $P < .01$) but not the TT genotype (1.5 ± 1.1; $P = .10$).

In contrast with the studies mentioned above, Kim et al. (2014) found no association between *COL5A1* rs12722 polymorphism and flexibility, assessed by Sit & Reach method and SRL (passive straight-leg-raise) methods, in Koreans.

Taken together, the existing studies confirm a role for *COL5A1* rs12722 polymorphism on the individual differences in ROM.

Finally, other polymorphisms in genes of collagen (*COL1A1* rs1800012, *COL3A1*rs1800255, *COL6A1*rs35796750, *COL12A1*rs970547, and rs240736) have been analyzed in two different studies (Bell et al., 2012; O'connell et al., 2013). The *COL1A1* rs1800012 and the *COL12A1* rs970547 polymorphisms were associated with joint laxity in 124 recreationally active subjects (Bell et al., 2012), while the *COL3A1* rs1800255, the *COL6A1* rs35796750, and the *COL12A1* rs970547 gene variants were not significantly associated with sit-and-reach, straight-leg-raise or total shoulder rotation ROM in a group of 350 apparently healthy and physically active Caucasians (O'connell et al., 2013).

13.3.2 ROMs and the *ACTN3* R577X genotype

Alongside the modifying role of *ACTN3* on sprint/power performance (Eynon et al., 2013; Papadimitriou et al., 2016) muscle mass/power (Zempo et al., 2010; Kikuchi et al., 2014) and muscle injury risk (Massidda et al., 2019), emerging evidence suggests this SNP may also impact flexibility and muscle stiffness.

a-Actinins are actin-binding proteins constituting the major structural component of the Z-line in myofibrils (Beggs et al., 1992). The 2 isoforms, a-actinin-2 and a-actinin-3, expressed in humans are encoded by their respective genes *ACTN2* and *ACTN3* (Mills et al., 2001). *ACTN3* is a fast-twitch specific isoform, expressed only in type II myofibers (Mills et al., 2001) and is reportedly important for anchoring actin and regulating the coordination of muscle-fiber contraction (Blanchard et al., 1989).

The first study on the role of *ACTN3* R577X polymorphism on flexibility has been carried out in 2014 by Kim et al. (2014) on Korean women, who reported that XX genotypes had decreased flexibility score in the sit-and-reach test.

Conversely, a couple of years later, two different studies on Japanese population reported an association between RR genotype and a decrease in flexibility score in the same test (Zempo et al., 2016; Kikuchi et al., 2017).

More recently, similar findings were obtained by Kikuchi et al. (2018) in a group of 52 Japanese men. The authors reported that carriers of the *ACTN3* RR genotype had lower ROM of elbow flexion than X-allele carriers at baseline, but the effect of *ACTN3* R577X genotype on this parameter was limited after isokinetic eccentric contractions (ECCs).

Joint flexibility may have been influenced by different factors other than the passive elastic properties of muscle fibers, for example, connective tissues surrounding the joint and muscle fibers and proprioceptive muscular tone (Gajdosik, 2001; Weerakkody et al., 2003). A recent study reported that single muscle fibers of a paraplegic patient harboring the RR genotype had higher hysteresis and Young's modulus than those harboring the RX and XX genotypes (Broos et al., 2012). Moreover, Seto et al. (2011) suggested that in the absence of a-actinin-3, increased the binding affinity of connectin (titin) to α-actinin-2 probably alters the concentration and organization of connection in the muscle fiber, thereby affecting the functional and structural properties of type II fibers.

In conclusion, the lack of consensus on the role of a-actinin-3 on flexibility is largely due to the small total studies number and their heterogeneity regarding the populations analyzed, in terms of ethnic background and gender, with greater clarity expected as research in the area evolves.

13.3.3 Genetic variations associated with muscle stiffness

Muscle stiffness is one of the main components of joint flexibility (Magnusson et al., 1997; Miyamoto et al., 2017) and it shows a sex-based variation; females exhibit lower muscle stiffness than males (Morse, 2011). Previous studies have shown that circulating levels of estrogen are negatively associated with muscle stiffness (Eiling et al., 2007; Bell et al., 2009) due to suppression of collagen synthesis (Kwan et al., 1996). The effects of estrogen on skeletal muscle are mediated via estrogen receptors (Brown et al., 2009; Wiik et al., 2009), and a recent study (Kumagai et al., 2019) hypothesized that two functional polymorphisms (rs2234693 C/T and rs9340799 G/A) in the estrogen receptor 1 gene (*ESR1*) could have a role in muscle stiffness variations among individuals.

The authors of the study investigated the association between two genetic polymorphisms in ESR1 and the prevalence of a history of muscle injury in 1311 top-level Japanese athletes. Moreover, the authors examined the differences in a physiological function, such as muscle stiffness, between two genetic polymorphisms (rs2234693 and rs9340799) of the *ESR1* gene in physically active subjects. These two functional polymorphisms, namely rs2234693 C/T and rs9340799 A/G, have been reported in the estrogen receptor 1 gene (*ESR1*), often identified by their restriction endonucleases

of *PvuII* (i.e., rs2234693) and *XbaI* (i.e., rs9340799). They are present in the first intron of *ESR1*, at positions 397 and 351 bp upstream of exon 2, respectively, and they influence the expression and the activation of the *ESR1* product, also altering the action of estrogen (Herrington et al., 2002).

The result of the study reported that the *ESR1* rs2234693 polymorphism was associated with muscle injury and stiffness, using elastographic methods to assess the ROMs. Specifically, the C allele was reportedly associated with resistance against muscle injury and a reduction in muscle stiffness compared with the T allele. *ESR1* rs9340799 was significantly associated with muscle stiffness of semitendinosus and semimembranosus only in female subjects. Such an approach would largely influence studies investigating associations among certain genetic polymorphisms and muscle stiffness and sport-related injury.

13.4 Genes and joint hypermobility in extreme phenotypes

13.4.1 Joint hypermobility (JH)

JH of a particular joint is defined by its ability to move beyond the average ROM, taking into account influencing factors such as age, sex, and ethnicity (Kirk et al., 1967; Jansson et al., 2004). JH is either inherited or acquired and the number, location, and severity of affected joints vary between individuals (Castori et al., 2017). JH has a strong genetic component, with female twin studies showing that at least 70% of the variance in phenotype is attributed to genetic factors (Hakim et al., 2004). Based on this information, the condition can be classified into the following groups (i) peripheral joint hypermobility (PJH), (ii) localized joint hypermobility (LJH), and the more commonly known, (iii) generalized joint hypermobility (GJH).

The hypermobility is then further characterized into a spectrum of conditions, which includes asymptomatic, symptomatic, and syndromic hypermobility (Castori et al., 2017). In these last conditions are included the Marfan syndrome (MFS) and the EDS, two hereditary disorders that provide a unique model for the study of the genetic basis of JH.

To date, the genetic basis of JH remains largely unknown and a recent GWAS, on detection and interpretation of shared genetic influences on 42 human traits, identified 18 loci associated with generalized joint laxity (Pickrell et al., 2016). In view of this, the genetic studies of the conditions mentioned above can actually give important insights into the mechanisms underlying JH, since JH is a prominent feature in these syndromes, albeit to a variable degree.

13.4.2 Assessment of Joint hypermobility (JH)

To establish whether a joint is hypermobile, its ROM is measured using a goniometer according to a set protocol and the results are compared to the normal

range (Juul-Kristensen et al., 2007). The Beighton score, which evaluates the mobility at five anatomic locations (thumb, wrist, elbow, and knee bilaterally and forward trunk flexion), is used as one of the tools to assess the type of JH on a scale from 0 to 9, where 1 point will be scored for hypermobility at each joint (Beighton and Horan, 1970). Children, adults, and the elderly with a score of $\geq$4, $\geq$5, and $\geq$6, respectively, are considered to have GJH.

An alternative tool for assessment is the 5-point questionnaire, which unlike the Beighton scores, allows the diagnosis of historical JH. This accounts for those who may have lost JH due to age, injury or other possible causes (Hakim and Grahame, 2003). Since these tools focus on specific joints, hypermobility of other joints should be assessed clinically.

13.4.3 Types of joint hypermobility

Unilaterally or bilaterally LJH can occur at small or large joints and would score $\geq$5 in adults and $\geq$6 in children using the Beighton score. Although LJH may be inherited, it can be acquired as a result of an injury, surgery, or training. GJH on the other hand, although it can also be acquired is more likely to be inherited. It usually involves the arms, legs, and axial skeleton and therefore, a person with GJH scores $\geq$5 in adults, $\geq$6 in children, and $\geq$4 in those above the age of 50.

PJH only presents in the hands and feet and differs to LJH as it involves all four limbs. These three basic categories can be further classified according to whether the individual has current or past hypermobility (historical), whether the hypermobility is asymptomatic or symptomatic or whether the hypermobility is a feature of a syndrome (Castori et al., 2017).

13.4.4 The spectrum

There is a spectrum of conditions containing JH. The hypermobility can be a feature of a syndrome mendelian disorder but hypermobility that presents when it is not part of such a syndrome that cannot be molecularly tested can be classified as part of the hypermobility spectrum (Castori et al., 2017):

(1) *Asymptomatic hypermobility*: any of the above types of hypermobility can exist with no secondary musculoskeletal manifestations (micro- and microtrauma, degenerative joint and bone disease, disturbed proprioception, muscle weakness, and musculoskeletal physical traits) or comorbidities and therefore such individuals are thought to have asymptomatic JH.

(2) *Symptomatic hypermobility*: symptomatic JH (LJH, PJH, or GJH) coexists with other musculoskeletal problems but is not associated with a genetic syndrome, such as EDS, and is part of the hypermobility spectrum disorders (HSD). Other organs may also be affected as well as the presence of some comorbidities, such as

gastrointestinal problems and pelvic and bladder dysfunction. In this case, the manifestations may also be due to historical hypermobility (H-HSD). Currently, there is no molecular basis for HSDs (Castori et al., 2017).

(3) *Syndromic hypermobility*: if an individual with GJH presents with either systemic manifestations or familial history of hypermobile type EDS (hEDS) exists, the individual can be considered for the diagnosis of hEDS, a syndrome which will be further discussed later in this section.

13.4.5 Heritable connective tissue disorders

To date, over 200 heritable connective tissue disorders have been described, most of which have diagnostic molecular tests available. Most genetic mutations associated with these disorders occur within genes encoding for structural components of the ECM or ECM regulating components. As a result, the connective tissue of the patient is affected, making these disorders multisystemic in nature. Therefore, connective tissue disorders can affect numerous organs (Castori et al., 2017; Meester et al., 2017).

13.4.5.1 Ehlers-Danlos Syndrome

EDS, is characterized by JH, skin extensibility and tissue fragility. Originally, this syndrome was thought to occur as a result of mutations in the collagen and collagen modifying genes, but the diagnosis has evolved to include a number of genetic mutations which may indirectly affect the collagen synthesis. The most recent nosology identifies 13 subtypes of EDS (Table 13.2; Malfait et al., 2017). For correct diagnosis of the EDS subtype, there is a set of major and minor criterion which must be met for each other. Further confirmations carried out by identification of the causal genetic variants.

JH is part of the major or minor criteria for all types with the exception of Brittle Cornea syndrome. Interestingly, the criteria does not always include GJH, but can also include PJH and historical JH. With the exception of the hypermobile-type, all types have been associated with genetic markers. The diagnostic criteria for hEDS were updated in the 2017 EDS nosology. The stricter criteria for diagnosis may help with the identification of genetic markers in future research.

13.4.5.2 Marfan syndrome

MFN was first described in 1896 (Marfan, 1886). Although the most distinguishable characteristic of individuals diagnosed with MFN is skeletal overgrowth, it is multisystemic in nature. Aortic dilatation and dissection are considered to be the most life-threatening, although other cardiac, ocular skeletal, and neurological manifestations can also occur. Consequently, patients diagnosed with MFN have decreased life expectancy (Murdoch et al., 1972; Silverman et al., 1995). MFN results from of abnormalities of fibrillin-1 protein, an important component of the ECM and a regulator of

Table 13.2 The 13 subtypes of EDS, the involved genes and the major clinical criteria (Castori et al., 2017).

EDS subtype	Gene	Major criteria examples
Classical EDS	*COL5A1, COL5A2*	Skin extensibility, joint hypermobility
Classical-like EDS	*TNXB*	Skin extensibility, joint hypermobility, easy bruising
Cardiac-valvular EDS	*COL1A2*	Severe cardiac valvular defects
Vascular EDS	*COL3A1*	Thin/translucent skin, congenital hip dislocation, skin hyperextensibility
Hypermobile EDS	Unknown	Skin extensibility, joint hypermobility, smooth velvety skin
Arthrochalasia EDS	*COL1A1, COL1A2*	Severe joint hypermobility, congenital hip dislocation, skin extensibility
Dermatosparaxis EDS	*ADAMTS2*	Extreme skin fragility, mild joint hypermobility, characteristic facial features
Kyphoscoliotic EDS	*PLOD1*, *FKBP14*	Kyphoscoliosis, joint laxity, muscle hypotonia
Brittle Cornea Syndrome	*ZNF469*, *PRDM5*	Thin cornea, keratoconus, keratoglobus, blue sclera
Spondylodysplastic EDS	*B4GALT7*, *B3GALT6*, *SLC39A14*	Short stature, muscle, bowing of limbs
Musculocontractural EDS	*CHST14*, *DSE*	Congenital contractures, characteristic craniofacial features, skin fragility/hyperextensibility
Myopathic EDS	*COL12A1*	Muscle hypotonia/atrophy, proximal joint contractures, distal joint hypermobility
Periodontal EDS	*C1R*, *C1S*	Severe periodontitis, lack of attached gingiva, pretibial plaques

transforming growth factor beta (TGFβ) signaling. These abnormalities are caused by mutations of the *FBN1* gene which are mostly inherited by the autosomal dominant pattern.

To date, over 1800 mutations have been identified (Dietz et al., 1991; Meester et al., 2017). Diagnosis is made using the revised Ghent nosology which focuses on the presence of aortic root enlargement and ectopia lentis (Loeys et al., 2010). Although genetic testing is not mandatory and therefore, is not used as the primary diagnostic tool, the latest nosology has greater emphasis in genetic testing. One of the reasons genetic testing is currently not compulsory for diagnosis is FBN1 mutations can be present without the classic Marfan phenotype. Therefore, the most recent nosology recommends only undergoing genetic testing when a positive phenotype is present to validate the diagnosis.

Genetic testing also plays an important role in differentiating between MFN and other connective tissue disorders. Currently, is it still under debate as to whether genetic testing should be carried out when only some symptoms of the syndrome are present (Radke and Baumgartner, 2014).

13.4.5.3 Loeys-Dietz syndrome

Loeys-Dietz syndrome was first described more recently in 2005. Although some features of MFN overlap with Loeys-Dietz syndrome, it is distinguishable by features such as hypertelorism, abnormal uvula (broad, with raphe or bifid), cleft palate and aortic and arterial aneurysm, and tortuosity (mostly head and neck vessels) (Loeys et al., 2005). Typically, the cardiac manifestations of Loeys-Dietz syndrome are more severe than those of MFN. For example, aortic aneurysms seem to dissect or rupture with a smaller diameter and at a younger age. Furthermore, these aneurysms are more widespread, therefore, they are not limited to the ascending aorta but tend to also affect aortic side branches and cerebral vessels (Loeys et al., 2006).

Other features which are seen in patients with Loeys-Dietz and not MFN include craniosynostosis, clubfoot, joint contractures, and cervical spine instability. Furthermore, symptoms such as inguinal, umbilical, and hiatal hernia as well as thin translucent skin, poor wound healing, and atrophic scars are seen in patients with Loeys-Dietz syndrome but not in MFN, although some of these features are similar to certain EDS subtypes features (Bradley et al., 2016). There are six subtypes of Loeys-Dietz syndrome.

The type of the syndrome is based on which TGFβ cytokine or receptor gene or SMAD gene is involved (Table 13.3; Van Hemelrijk et al., 2010). Currently, there are no formal diagnostic criteria and diagnoses are based on the presence of aortic aneurysms or dissection as well as the identification of the involved gene. Family history can also be considered. Although, it must be taken into account that Loeys-Dietz syndrome has been observed as a nonpenetrance trait and intrafamilial variation has been observed in Loeys-Dietz syndrome families and requires further exploration (Loeys et al., 2006).

Table 13.3 The Loeys-Dietz syndrome (LDS) subtypes and the involved genes.

LDS subtype	Gene
LDS type 1	*TGFBR1*
LDS type 2	*TGFBR2*
LDS type 3	*SMAD3*
LDS type 4	*TGFB2*
LDS type 5	*TGFB3*
LDS type 6	*SMAD2*

13.5 Conclusions

This chapter focuses on genetic polymorphisms of flexibility (ROMs and muscle stiffness) and mutations of JH in extreme phenotypes (EDM, MFN, and LDS).

Although it is well known that flexibility is influenced by environmental factors, a recent metaanalysis showed that half of the variance in flexibility is explained by genetic factors (Schutte et al., 2016), such as certain genetic polymorphisms including in the genes *COL5A1*, *ACTN3*, and *ESR1*. Nevertheless, there are few studies which examined the effects of genetic polymorphisms on flexibility. In addition, flexibility is influenced by several factors such as joint structure, ligaments, tendons, muscles, skin, fat tissue, history of tissue injuries, body temperature, activity level, age, sex, and so on. Therefore, it is necessary to examine the effects of genetic polymorphisms on flexibility under well-controlled conditions. Current studies of flexibility-associated polymorphisms have revealed the polygenic profile for determining flexibility; however, additional studies are needed to explain the genetic components.

JH is a complex trait as it occurs on a spectrum of severity which can include comorbidities and can either be acquired or inherited. Currently, more research is required to understand the molecular basis of the asymptomatic and symptomatic JH (hypermobility spectrum disorders). GWAS may be a useful tool to better our understanding of the genetic basis of generalized JH. To the best of our knowledge, only one GWAS has been carried out in the field identifying 18 loci associated with generalized joint laxity and demonstrating the complexity of the trait (Pickrell et al., 2016).

More research exists investigating genetic syndromes which are associated with syndromic hypermobility, such as EDS, MFN, and LDS syndromes. Such syndromes are usually as a result of genetic mutations in ECM or ECM regulating components. Although the molecular basis of these syndromes is well researched, this is still an emerging field. Future research is necessary to further our knowledge in this field as well as improve the diagnostic tools.

Finally, further studies in exercise and sports science should include more detailed analyses of genetic polymorphisms including whole genome sequencing, exome sequencing, and/or GWAS to uncover genetic polymorphisms and/or mutations for determining flexibility and JH.

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CHAPTER FOURTEEN

Genetics of muscle fiber composition

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14.1 Introduction

Human skeletal muscle is an extremely heterogeneous tissue that is composed of type I (slow twitch) and type II (fast twitch) muscle fibers. Type II fibers can be further categorized into subtypes of IIa and IIx fibers, which are also known as fast twitch oxidative and fast twitch glycolytic fibers, respectively. It has previously been reported that large interindividual differences in the fiber composition of human skeletal muscle (i.e., 15%–85% type I fibers, 5%–77% type IIa, and 0%–44% type IIx) exist in the general population. These differences in the fiber composition of skeletal muscles partly explain the marked differences in physical performance between individuals, such as endurance-oriented or sprint-oriented athletic performance, and occurrence of lifestyle-related diseases such as obesity, dyslipidemia, type 2 diabetes mellitus, and hypertension. It is possible that environmental factors such as exercise training not only induce metabolic adaptation but also limit fiber-type transformations. This implies the involvement of genetic factors in determining human muscle fiber composition. An early study involving twins reported that heritability estimates for determining muscle fiber composition were almost 100% in men and 93% in women. Based on these findings, it was commonly believed that muscle fiber composition was influenced only by genetic factors. Subsequently, other researchers showed that heritability estimates for muscle fiber composition was lower, but probably more than half, using relatively large samples. These findings indicate that genetic factors exert a greater effect than environmental factors, or that both of these factors exert comparable effects for determining muscle fiber composition. Thus, genetic factors are suggested to play an important role in determining human skeletal muscle fiber composition. Therefore, it is necessary to examine which specific genetic factors contribute to muscle fiber composition. The substantial genetic contribution to human muscle

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fiber composition, and its related phenotypes, such as physical performance and/or lifestyle-related diseases, indicate the possibility of using genetic approaches to individualize training approaches for enhancing competitive athletic performance, or even to help select appropriate athletic disciplines by using certain genetic polymorphisms (i.e., talent identification and/or talent transfer to other events). In this chapter, we focus on selected genetic polymorphisms as likely partial determinants of human skeletal muscle fiber composition.

14.2 Function of skeletal muscle fibers

Previously, muscle fiber types were classified as type I and II, with subgroups IIa and IIb on the basis of myosin ATPase histochemical staining (Brooke and Kaiser, 1970). Immunohistochemical staining with antibodies specific for different myosin heavy chain (MHC) isoforms and in situ hybridization analyses which aimed to detect MHC transcripts showed that human IIb fibers correspond, in fact, to IIx fibers, containing a MHC IIx similar to that present in type IIx fibers of mouse and rat muscle, whereas MHC IIb is not expressed in human skeletal muscle (Smerdu et al., 1994). Thus, human skeletal muscle contains type I, IIa, and IIx fibers, though human IIx fibers used to be referred to as type IIb. Type IIa, IIb, and IIx fibers are contained in skeletal muscle of other mammals such as rodents. As shown in Table 14.1, these

Table 14.1 Characteristics of three different fiber types.

Characteristic	Type I	Type IIa	Type IIb/IIx
Alternate name	Slow oxidative (SO) Slow-twitch (ST)	Fast oxidative-glycolytic (FOG) Fast-twitch A (FT-A)	Fast glycolytic (FG) Fast-Twitch B (FT-B)
Color	Red	Pink	White
Contraction velocity	Slow	Intermediate	Fast
ATPase capacity	Slow	Intermediate	Fast
Force production	Low	Intermediate	High
Fatigue resistance	High	Intermediate	Low
Oxidative capacity	High	Intermediate	Low
Glycolytic capacity	Low	Intermediate	High
Mitochondrial density	High	Intermediate	Low
Myoglobin content	High	Intermediate	Low
Capillary density	High	Intermediate	Low
Motor unit	Small	Intermediate	Large
Metabolism	Oxidative (aerobic)	Oxidative and glycolytic (combined)	Glycolytic (anaerobic)

fiber types differ in maximal velocity of muscle shortening, type I showing the slowest contraction properties and type IIx the fastest, as determined by studies involving human single muscle fibers with defined MHC composition, due to activity of myosin ATPase (Bottinelli and Reggiani, 2000; Larsson and Moss, 1993). When combined with a larger mean cross-sectional area (at least in males), the greater maximal velocity of muscle shortening of type II, and especially type IIx fibers, means that they can produce substantially greater maximal power compared with type I fibers (Bottinelli and Reggiani, 2000; Larsson and Moss, 1993; Staron et al., 2000). Indeed, power (rather than strength or size per se) is frequently a major factor in determining sporting success at one extreme of the human performance continuum, and functional ability in daily tasks at the other extreme of performance (Wilson et al., 1993). This is because few sporting or daily tasks involve muscle contractions at the extremes of the force-velocity relationship where either the speed of movement is zero (i.e., a maximal isometric contraction, where power produced is zero) or the force produced is zero (i.e., a maximal velocity contraction, where power production is again also zero). Enzymic histochemical studies showed that type I fibers are rich in oxidative enzymes but relatively poor in glycolytic enzymes, whereas type IIx fibers have high glycolytic enzyme activity and low levels of oxidative enzymes, and type IIA fibers have intermediate properties (Essen et al., 1975). Similar conclusions were reached with microchemical analyses of single fibers defined with myosin ATPase (Hintz et al., 1984).

Since skeletal muscle is highly metabolically active, accounts for ~23% of total resting energy expenditure (Gallagher et al., 1998) and makes up a high proportion (i.e., 30%–40%) of total body mass (Janssen et al., 2000) and strongly influences overall metabolism. Type I fibers and oxidative muscle fibers utilize primarily fatty acids and frequent use of these muscle fibers leads to a reduction in fat mass and improvement in insulin sensitivity (Kriketos et al., 1996; Toft et al., 1998). Variability in the proportion of skeletal muscle fibers has been investigated and was found to contribute to susceptibility and aspects of chronic diseases such as obesity, type 2 diabetes, and hypertension (Bassett, 1994; Wade et al., 1990). In addition, 40% of the variability in body fat percentage could be explained by skeletal muscle fiber composition (Wade et al., 1990). Accordingly, a low percentage of type I muscle fibers was shown to be a risk factor for the development of obesity and insulin resistance (Gerrits et al., 2010; Lillioja et al., 1987; Sun et al., 2002; Tanner et al., 2002). Additionally, a previous study reported that hypertensive subjects had a tendency to possess a higher proportion of type II fibers (Frisk-Holmberg et al., 1983). Furthermore, the percentage of type IIx fibers was related to diastolic blood pressure in normotensive men and to mean blood pressure in hypertensive subjects (Hernandez et al., 2001). Thus, human skeletal muscle fiber composition influences chronic diseases such as obesity, type 2 diabetes, and hypertension, as well as physical performance.

14.3 Heritability estimates of skeletal muscle fiber composition

An important question in biology is whether observed variation (i.e., inter-individual differences) in a particular phenotype is due to genetic factors or to environmental factors, sometimes presented as the "nature (genetics) versus nurture (environment)" debate. Heritability estimates indicate what proportion of variation in certain phenotypes is due to genetic factors versus environmental ones. These estimates range from 0.0 (0%) to 1.0 (100%), with 0.0 indicating that genetic factors are not a contributing factor at all, and 1.0 indicating that genetics is the only factor. A meta-analysis of heritability estimates of human phenotypes in studies involving twins was conducted on variance components for 17,804 phenotypes from 2748 published studies, including 14,558,903 partly dependent twin pairs (Polderman et al., 2015). Based on this meta-analysis, heritability estimates across all phenotypes is approximately 50%. Thus, 50% of the variance in various human phenotypes can be explained by genetic factors and the remaining 50% by environmental factors.

It is commonly believed that human muscle fiber composition is genetically determined (i.e., nature is important), changing little as a result of environmental factors such as physical training (i.e., nurture has almost no effect whatsoever). Indeed, Komi et al. (1977) reported that heritability estimates of muscle fiber composition were almost 100% for men and 93% for women, although the sample size was small and there were other methodological limitations. In brief, skeletal muscle fiber composition was investigated in 31 pairs of male and female monozygotic and dizygotic twin pairs, aged between 15 and 24 years. Fiber type was analyzed using ATPase in biopsy samples taken from the vastus lateralis muscle. Percentages of type I fibers were almost identical between monozygotic twins, whereas they were quite variable between dizygotic twins. In addition, physical training such as chronic endurance and/or resistance exercise does not seem to lead to a conversion between type I and type II fibers; however, some changes do occur within type II fibers, mainly between types IIa and IIx (Hamel et al., 1986; Aagaard et al., 2011).

This early study could not be replicated in other studies using relatively large samples (Bouchard et al., 1986; Simoneau and Bouchard, 1995). Bouchard and coworkers originally found heritability estimates that were markedly lower using a relatively large number of subjects, namely, nontwin brothers ($n = 32$ pairs), dizygotic twins ($n = 26$ pairs), and monozygotic twins ($n = 35$ pairs). They used a needle biopsy of the vastus lateralis to determine muscle fiber composition (I, IIa, IIx). For type I fiber composition, intraclass correlations were 0.33, 0.52, and 0.55 in brothers, dizygotic twins, and monozygotic twins, respectively. Although both brothers and dizygotic twins share 50% of their genes, intraclass correlation was different between brothers and dizygotic twins compared with between dizygotic twins and monozygotic twins. Because dizygotic twins experience more similar environmental factors than nontwin brothers, these results suggested that

skeletal muscle fiber composition was almost entirely determined by environmental factors. However, Simoneau and Bouchard (1995) reanalyzed the heritability estimates using expanded monozygotic twins data and revealed a relatively high contribution involving genetic factors. They concluded that genetic factors (approximately 45%) contributed more to the determination of skeletal muscle fiber composition than environmental factors (approximately 40%), with the remaining 15% attributed to muscle sampling and technical variance, as shown in Fig. 14.1. Thus, twin studies to calculate heritability estimates have certain methodological problems, including small sample sizes. However, genetic factors appear to exert a greater effect than environmental factors or both exert comparable effects in determining muscle fiber composition.

14.4 Effect of age, gender, and ethnicity on muscle fiber composition

14.4.1 Effect of age

In humans, muscle strength and mass decrease with age from peak values observed between 20 and 30 years old. Two of the possible causes of this remarkable reduction are aging-induced muscle fiber atrophy and muscle fiber-type transitions. Generally, the cross-sectional area of muscle fibers increases from 5 to approximately 20 years of age and starts to decrease from the age of around 25 (Lexell et al., 1988). It has been suggested that muscle fiber composition differs with age, that is, children show a higher percentage of type I fibers than newborns and adults. Newborn babies and infants aged

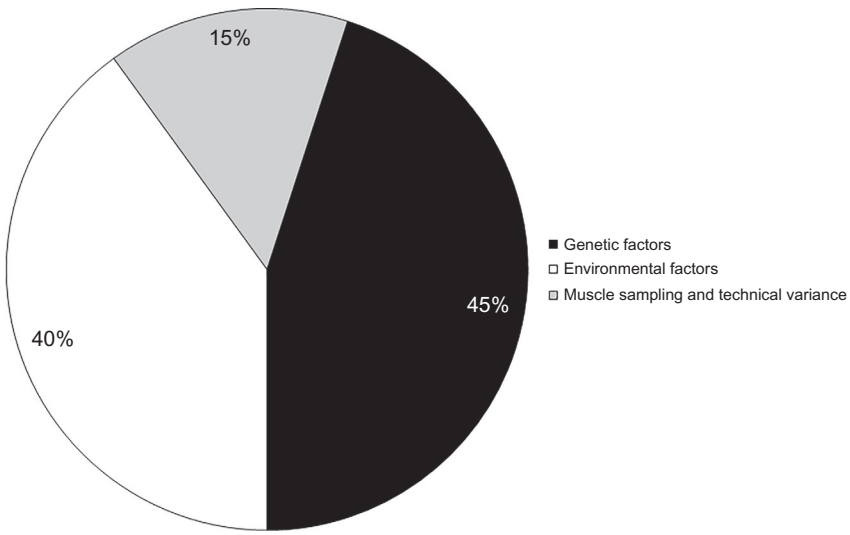

Fig. 14.1 Factors for determinants of muscle fiber composition.

0–2 years tend to have ~50% of type I fibers, and 10%–17% of the fibers are classified as undifferentiated muscle fibers, termed type IIc (Elder and Kakulas, 1993), whereas children aged 2–5 years old tend to exhibit ~70% type I fibers (Lexell et al., 1988; Oertel, 1988). Thereafter, the proportion of type I fibers gradually decreases between 5 and 20 years of age, which may be caused by the transition from type I to type II fibers (Elder and Kakulas, 1993; Lexell et al., 1988; Oertel, 1988). Thus, skeletal muscle fiber composition may be changeable to some extent; at least during youth, and the proportion of type I fibers appears highest at around 2–5 years old.

In adults, type I fibers gradually increase and type II fibers gradually decrease (Hortobagyi et al., 1995; Korhonen et al., 2006; Kumagai et al., 2018). Indeed, type I fibers were found to be increased with age by using cross-sectional studies involving broad age ranges in humans (Hortobagyi et al., 1995; Korhonen et al., 2006). Recently, our group also confirmed these trends with over 100 adult male individuals, but not with over 100 adult females (Kumagai et al., 2018) (Fig. 14.2). In support of these findings, a longitudinal study in elderly male subjects shows that type IIx fibers significantly decrease during an 11 year follow-up period (Aniansson et al., 1992). In contrast, another follow-up study using young male and female individuals suggests that there is a gender

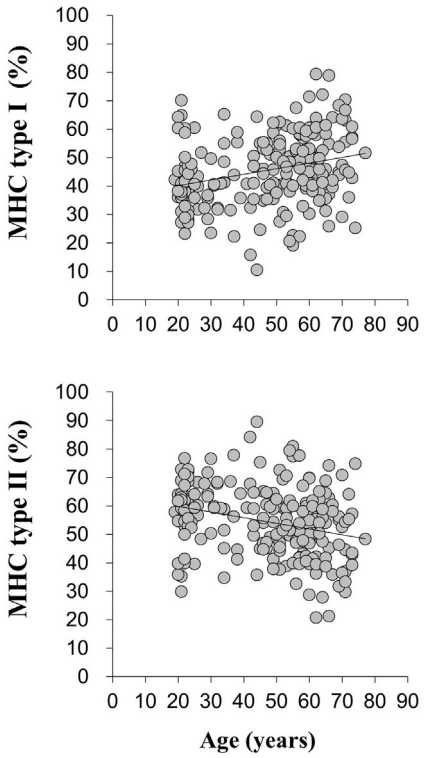

Fig. 14.2 Relationship between age and MHC type I muscle fiber composition by gender.

difference for aging-induced muscle fiber transition, that is, type I fibers decrease with age in male subjects, whereas they increase with age in female subjects (Glenmark et al., 1992). Taken together, although several studies have suggested that aging shifts muscle fiber composition from type II to type I fibers, it is necessary to consider sex difference (male or female), age (young or old age), and measurement (number of muscle fibers, cross-sectional area or MHC isoforms) in a future study.

14.4.2 Effect of sex difference

In humans, there are many gender-based differences such as appearance, body composition, physical fitness, and athletic performance, as well as health-related traits such as incidence of cardiovascular disease, type 2 diabetes. Sprint athletes obviously require a proliferation of type II muscle fibers, and 70%–80% of the muscle fibers in skeletal muscles of elite-level sprint athletes are type II muscle fibers (Andersen et al., 2000). Contradictorily, endurance athletes clearly require a proliferation of type I muscle fibers. The data involving Japanese athletic records indicates that there are 5%–16% differences between male and female Japanese records in track and field events. The sex differences in track and field are the greatest in 400 m events and minimal in 100 km events, and the differences are approximately 16% and 5%, respectively. Interestingly, the gender difference in track and field events gradually decreases with increasing total distance from 400 m to 100 km events. Obviously, short-distance events require anaerobic ability and long-distance events require aerobic capacity. These data demonstrate that females are relatively well suited to long-distance events than males and imply that females exhibit higher proportions of type I fiber than males.

Several previous studies have examined muscle fiber composition and included data for both male and female subjects (Table 14.2). Although small studies show that the percentage of slow twitch fibers is higher in male than female subjects, or with no significant differences, most of the large studies reported by Simoneau and Bouchard, Starone and colleagues, Glenmark et al., and Simoneau and coworkers demonstrate that female subjects show higher percentages of slow twitch fibers than male subjects (Glenmark et al., 1992; Simoneau and Bouchard, 1989; Simoneau et al., 1985; Staron et al., 2000). To support these previous results, we recently reported that there were sex differences in muscle fiber composition as measured by MHC isoforms (Fig. 14.3) (Kumagai et al., 2018). The proportions of type I fiber are $40.5 \pm 11.7\%$ in males and $50.3 \pm 11.1\%$ in females, type IIa are $35.8 \pm 8.3\%$ in males and $30.8 \pm 8.2\%$ in females, and type IIx are $23.6 \pm 9.2\%$ in males and $19.0 \pm 8.3\%$ in females. The distribution of female subjects is shifted to the higher percentage area (right side) compared with male subjects as shown in Fig. 14.3. These results indicated that slow twitch fiber composition was higher in female than male individuals and that the proportion of fast twitch fibers was higher in males than females, which may influence sex-based differences in physical fitness and athletic performance.

Table 14.2 Sex difference in MHC type I fiber compositions.

Reference	Male subjects	Female subjects	Difference
Simoneau and Bouchard (1989)	46 ± 15 $n = 215$	51 ± 13* $n = 203$	Female > male
Kumagai et al. (2018)	40.5 ± 11.7 $n = 102$	50.3 ± 11.1* $n = 109$	Female > male
Staron et al. (2000)	33.9 ± 11.4 $n = 95$	41.0 ± 12.9* $n = 55$	Female > male
Glenmark et al. (1992)	48 ± 13 $n = 55$	55 ± 12* $n = 28$	Female > male
Simoneau et al. (1985)	36.9 ± 10.7 $n = 37$	42.7 ± 11.8* $n = 38$	Female > male
Essen-Gustavsson and Borges (1986)	58 ± 15 $n = 34$	51 ± 10* $n = 31$	Male > female
Komi and Karlsson (1978)	55.9 ± 11.9 $n = 40$	49.1 ± 7.7* $n = 22$	Male > female
Oertel (1988)	41 ± 10 $n = 18$	43 ± 9 $n = 19$	NS

*$P < .05$ versus male subjects.
Data are shown mean ± SD.

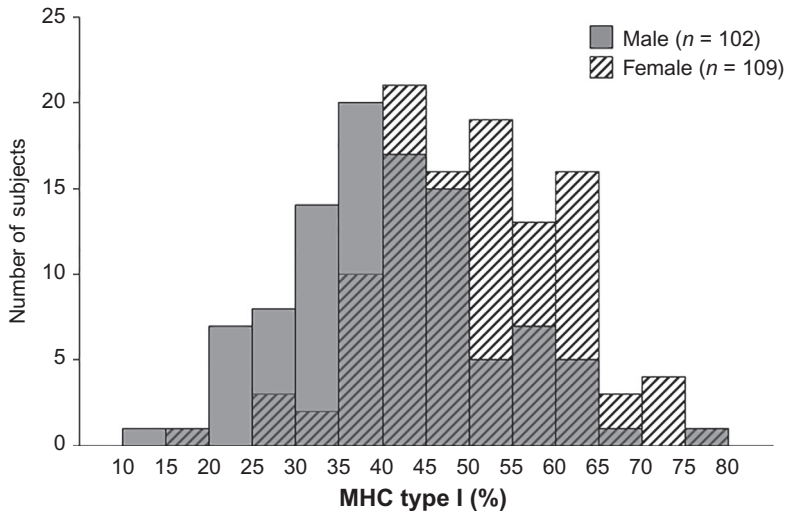

Fig. 14.3 The number of individuals with each proportion of MHC type I muscle fiber in males and females.

14.4.3 Effects of ethnicity

Ethnicity, a category of population based on ancestry, is mainly affected by genetic differences. Ethnicity is associated with a number of phenotypes such as obesity and disease risk, among others, as well as physical performance. For example, Caucasian populations have a lower risk of diabetes and higher aerobic capacity compared to African ancestries (DeLany et al., 2014; Duncan et al., 2005; Wang et al., 2010). Regarding ethnic differences, one of the most plausible scenarios involving the relationship between ethnicity and physical performance is a final race for 100 m in track and field events in international games such as the Olympics and World Championships. Probably, the origin of the most finalists in 100 m events is from "West African ancestry." Indeed, the top 10 fastest 100 m sprinters in the world are of West African ancestry such as African-American and/or Jamaican. Therefore, it is possible that ethnicity influences muscle fiber composition. Several previous studies demonstrated that the proportion of type I fibers is higher in Caucasian populations than in those of African ancestry (Ama et al., 1986; DeLany et al., 2014; Tanner et al., 2002). In contrast, the proportion of type II fibers (i.e., the sum of type IIa and type IIx) is higher in individuals of African ancestry than in Caucasian populations (Ama et al., 1986; DeLany et al., 2014). Although there are no significant associations, the data reported by Duey and colleagues show similar trends to other studies (Duey et al., 1997). In addition, Khon et al. also reported that Caucasian endurance runners exhibit higher percentages of type I fibers and lower proportions of type IIa fibers than runners of South African descent (Kohn et al., 2007), although this study did not compare Caucasian and East African endurance runners. According to these previous data, African populations, at least those of West African ancestry, may show higher proportions of type II fibers than Caucasian populations, which might influence high sprint performance. Unfortunately, there is no study which compared muscle fiber composition among Asian, European, and African populations.

14.4.4 Effects of genetic polymorphisms on muscle fiber composition

14.4.4.1 ACTN3 *(rs1815739)*

α-Actinin-2 and -3 proteins are localized to the Z-disk in skeletal muscle and help to anchor actin filaments, which play an important role in contracting a skeletal muscle or maintaining its structure. α-actinin-2 is expressed in all skeletal muscle fibers, whereas α-actinin-3 is expressed only in skeletal muscle type II (i.e., fast twitch) fibers (North and Beggs, 1996). Previous study reported that skeletal muscle-specific α-actinin-3 gene (*ACTN3*) knockout mice display reduced force generation and fast fiber diameter, increased activity of aerobic enzymes, and enhanced recovery from fatigue, suggesting a shift in the properties of fast fibers toward those characteristic of slow fibers (MacArthur et al., 2007, 2008). Thus, the expression status of *ACTN3* in skeletal muscle might influence muscle fiber composition.

In humans, *ACTN3* is located on chromosome 11 and contains 22 exons. A common genetic polymorphism involving codon 577 of *ACTN3* causes an amino acid substitution of arginine (R or Arg) for a premature stop codon (X or TER), that is, R577X or Arg577Ter, by C-to-T transition in the 16th exon. Homozygosity for the common nonsense polymorphism R577X in *ACTN3* results in complete deficiency of α-actinin-3 protein in type II muscle fibers. Experiments in knockout mice showed that ACTN3 deficiency affects skeletal muscle function (Berman and North, 2010). Therefore, ACTN3 may play an essential functional role in type II muscle fibers.

Yang and coworkers originally reported that individuals with the RR + RX genotype involving the R577X polymorphism of *ACTN3* were found more frequently in elite Australian sprint/power athletes than in controls (Yang et al., 2003). This finding has been replicated in a broad variety of ethnic groups (Druzhevskaya et al., 2008; Eynon et al., 2009; Kikuchi et al., 2015; Mikami et al., 2014; Niemi and Majamaa, 2005; Roth et al., 2008). In a meta-analysis, the RR genotype and/or R allele of *ACTN3* was found to be more common among sprint/power athletes than in controls (Alfred et al., 2011; Ma et al., 2013). This R577X polymorphism induces ACTN3 deficiency in type II muscle fibers and may elevate the percentage of slow-twitch fibers by activating calcineurin signaling (Frey et al., 2008; Seto et al., 2013). There is some evidence that the activation of calcineurin/NFAT specifically induces a muscle fiber-type switch toward a slow twitch and oxidative phenotype, with an increase in the expression of a subset of genes associated with type I myofibers, such as myoglobin and troponin I slow skeletal muscle (5, 6), allowing for sustained and fatigue-resistant muscle activity. Indeed, previous studies have demonstrated that the proportion of type IIx fiber in the vastus lateralis is higher in subjects with *ACTN3* RR and RX genotypes than in those with XX genotype in healthy young males (Ahmetov et al., 2011; Kumagai et al., 2018; Vincent et al., 2007). However, no association was found between the *ACTN3* R577X polymorphism and muscle fiber composition in female subjects (Kumagai et al., 2018). Therefore, the effect of this *ACTN3* polymorphism on muscle fiber composition may be different between male and female subjects.

14.4.4.2 ACE *(rs1799752)*

Angiotensin-converting enzyme (ACE) is a pivotal component of the renin-angiotensin system (RAS) and plays a critical role in circulatory homeostasis by causing blood vessels to constrict by converting angiotensin I to angiotensin II. This enzyme is also regulated in glucose metabolism by bradykinin, which induces glucose uptake by muscle cells. In addition, local RASs, in a variety of tissues, promote cell growth (Puthucheary et al., 2011) in diverse tissues, including in lung and skeletal muscles (Jones and Woods, 2003). ACE inhibitors are widely used as pharmaceutical drugs for the treatment of cardiovascular diseases such as hypertension.

In humans, the *ACE* gene is located on chromosome 17 and contains 26 exons. A polymorphism involving human *ACE* has been identified which consists of either the presence (insertion, I) or absence (deletion, D) of a 287 base pair (bp) Alu repeat sequence (rs1799752 or rs4340) in intron 16, which is associated with both circulating and tissue levels of ACE (Danser et al., 1995; Rigat et al., 1990). Higher ACE activity is associated with the D allele in both Caucasian and Asian populations, but not in African populations.

The ACE I/D polymorphism was first reported to impact on human physical performance (Montgomery et al., 1998). Subsequently, several case-control association studies have reported that the *ACE* I/D polymorphism is associated with physical performance; in particular, I allele carriers show lower ACE serum concentrations and greater success in endurance-related sporting performance (Montgomery et al., 1998; Myerson et al., 1999). Despite the consistency of such findings, data involving East Asian populations from various groups have reported conflicting results, namely, that the *ACE* I allele is associated with sprint/power performance and that the *ACE* D allele is associated with endurance performance (Kim et al., 2010; Tobina et al., 2010; Wang et al., 2013). In addition to these findings, the *ACE* I allele is also associated with handgrip strength in elderly Japanese (Yoshihara et al., 2009). Regarding muscle fiber composition, we demonstrated that the *ACE* D allele is associated with a higher proportion of type I muscle fibers than the *ACE* I allele in Japanese male adults, even after adjustment for age and BMI (Kumagai et al., 2018). This result supports findings of an association between *ACE* I/D polymorphism and physical performance in Asian populations. Nevertheless, the findings from one group using Japanese individuals support similar observations in European populations. Although further extensive studies are necessary to conclude any association of *ACE* I/D polymorphism on muscle fiber composition and physical performance, the effect of the I/D polymorphism involving the *ACE* gene may be different in European and Asian populations.

14.4.4.3 HIF1A *(rs11549465)*

Hypoxia-inducible factor 1 (HIF-1) is found in mammalian cells cultured under low O_2 concentrations and is a transcription factor regulating the expression of genes providing cell adaptation to hypoxia (Wang et al., 1995). The HIF-1 encoding gene, *HIF1A*, interacts closely with processes such as glycolysis (lactate dehydrogenase, phosphofructokinase, pyruvate kinase genes), glucose transport (GLUT family glucose transporter genes), and angiogenesis (erythropoietin, vascular endothelium growth factor, VEGF, and VEGF receptor genes) (Airley and Mobasheri, 2007; Iyer et al., 1998; Semenza et al., 1996; Wang et al., 1995). *HIF1A* is expressed in almost all tissues and its expression is higher in type II fibers than in type I fibers (Pisani and Dechesne, 2005). Thus, *HIF1A* might control muscle fiber composition.

In humans, *HIFIA* is located on chromosome 14 and contains 16 exons. There is a common genetic polymorphism (rs11549465) involving this gene, which causes amino acid replacement from the proline (Pro) to serine (Ser) at position 582 of the codon (Pro582Ser) due to a C-to-T transition in the 12th exon. This polymorphism is located in an oxygen-dependent degradation domain of HIF-1α, which is related to HIF-1α function (Clifford et al., 2001). Indeed, in an in vitro study, carriers of the Ser polymorphism exhibited the higher transcriptional activity of *HIF-1α* compared with Pro/Pro carriers under normoxic and hypoxic conditions (Tanimoto et al., 2003). It has been previously reported that individuals with the Pro/Ser genotype are associated with an increased proportion of type II muscle fibers of the vastus lateralis compared to individuals with the Pro/Pro genotype in Russian speed skaters (Ahmetov et al., 2008), although this finding was not replicated in another study conducted in a sedentary Japanese population (Kumagai et al., 2018). Nevertheless, *HIF-1α* knockout mice exhibit a higher proportion of type I fibers, as well as a metabolic shift from glycolysis toward oxidation (Mason et al., 2004). Furthermore, HIF-1α overexpression in mice revealed a higher proportion of type II fibers. Thus, HIF-1α induces type 2 fiber transformation to the fast twitch muscle fibers in mice (Lunde et al., 2011). Taken together, the Pro582Ser polymorphism involving HIFIA might influence muscle fiber composition, at least in European athletes. However, further replication studies in varying ethnicities are necessary to conclude this association between the HIFIA Pro582Ser polymorphism and muscle fiber composition in humans.

14.4.4.4 KDR *(rs1870377)*

Kinase insert domain receptor (KDR) is also known as tyrosine kinase growth factor receptor, vascular endothelial growth factor receptor (VEGFR), or vascular endothelial growth factor receptor 2 (VEGFR2). Vascular endothelial growth factor (VEGF) is described as a growth factor for vascular endothelial cells (Ferrara et al., 1992), which plays essential roles in the regulation of angiogenesis, vascular development, vascular permeability, and embryonic haematopoiesis. VEGF expression is upregulated by hypoxia, and its cell-surface receptor, KDR, is exclusively expressed by endothelial cells. The levels of *VEGF* and *KDR* gene transcripts are correlated with the normal development of the ocular vasculature in humans. Because it has been reported that the VEGF-KDR pathway is a proangiogenic factor considered to be central to capillary growth in skeletal muscle (Egginton, 2009), its actions might influence muscle fiber type.

In humans, *KDR* is located on chromosome 4 and contains 30 exons. There is a common genetic polymorphism (rs1870377) of this gene which causes an amino acid replacement from glutamine (Gln or Q) to histidine (His or H) at position 472 of the coding region (Gln472His or Q472H) by A-to-T transversion in the exon 11. This polymorphism is located at the third and fifth NH_2-terminal Ig-like domain within the extracellular region, which plays an important role in binding certain ligands. Indeed, a previous

study reported that the His472Gln polymorphism in KDR influences the efficiency of VEGF binding to KDR (Wang et al., 2007). In addition, *KDR* null mutant mice were observed to be embryonically lethal. Therefore, it may be worth exploring the effects of functional polymorphisms within this candidate gene on muscle fiber type. It has been previously reported that individuals who are Gln carriers (i.e., sum of Gln/His and Gln/Gln genotypes) are associated with an increased proportion of type I muscle fibers of the vastus lateralis muscle compared to individuals with the His/His genotype in both Russian speed skaters and controls (Ahmetov et al., 2009). However, this finding was not replicated in another study in a Japanese sedentary population (Kumagai et al., 2018). Likewise, Gln carriers of this polymorphism are more likely found in elite Russian endurance athletes, but not elite Japanese endurance athletes. So far, this genetic polymorphism is a candidate for determining muscle fiber type in European populations (Ahmetov et al., 2009; Yvert et al., 2016).

14.4.4.5 AGTR2 *(rs11091046)*

Angiotensin II receptor, type 2 (AGTR2) is also known as the AT2 receptor, which is a protein encoded by *AGTR2*. AGTR2 plays an important role in cardiovascular function and muscle growth, which are mediated by the RAS (Fyhrquist and Saijonmaa, 2008; Jones and Woods, 2003; Johnston et al., 2011). Circulating angiotensinogen (AGT), produced by the liver, is cleaved by renin to yield angiotensin I, and further cleaved to angiotensin II by ACE. Angiotensin II is the major effector molecule of the RAS, acting via angiotensin II type 1 receptor (AGTR1) and AGTR2. Angiotensin II, among several functions, exerts effects on muscle performance, for example, a direct hypertrophic effect as a skeletal muscle growth factor, enhanced noradrenaline release from the nervous system, and increased capillary density in skeletal muscle, and has previously been shown to be associated with strength- and power-related sports (Jones and Woods, 2003; Puthucheary et al., 2011). AGTR2 mediates the effects of angiotensin II on cellular differentiation and growth. It is generally reported to have opposite effects to those mediated by AGTR1 (Fyhrquist and Saijonmaa, 2008), which is involved in the regulation of muscle development, that is, reducing muscle weight, amount of fiber, and type II fiber area (Zempo et al., 2016).

In humans, *AGTR2* is located on the X chromosome and contains three exons. There is a common C/T genetic polymorphism (rs11091046) of this gene which is located within the 3′-untranslated region of *AGTR2*, at a microRNA-response element position, possibly corresponding to the hsa-miR-208a-5p and hsa-miR-208b-5p binding sites (Yvert et al., 2018). It has been reported that miRNA-208a and -208b are encoded by the myosin heavy chain 6 (*MYH7*) and 7 (*MYH7*) genes in humans. Individuals with the C allele were shown to be associated with an increased proportion of type I muscle fibers of the vastus lateralis muscle, compared to individuals with the A allele, in both Russian speed skaters and controls (Mustafina et al., 2014), although this finding was not replicated in another study in a Japanese sedentary population (Kumagai

et al., 2018). Thus, *AGTR2* gene products can be influenced partly by miR-208 a- and/or b-5p, and that the C allele of the *AGTR2* rs11091046 polymorphism in this region can reduce miRNA/mRNA binding affinity, leading to an increased number of AGTR2 products playing possible roles in muscle fiber composition. In addition, the A allele of this polymorphism is more likely to be identified in elite Russian and Brazilian power athletes (Mustafina et al., 2014; Guilherme et al., 2018), whereas the opposite was found in elite Japanese and Russian sprinters (Yvert et al., 2018). Further extensive studies are necessary to draw conclusions regarding the effects of the *AGTR2* rs11091046 polymorphism on muscle fiber composition.

14.4.5 Combined effects of ACTN3 R577X and ACE I/D in the Japanese population

Since certain genetic polymorphisms are suggested to play an important role in determining skeletal muscle fiber composition, as described above, it is necessary to investigate the combined effects of genetic polymorphisms on the proportion of muscle fiber composition. Genetic polymorphisms such as ACTN3 R577X and the ACE I/D are the most famous and well-studied genetic polymorphisms surrounding sports performance. We recently demonstrated the combined effects of these genetic polymorphisms on muscle fiber composition in a Japanese population (Kumagai et al., 2018). Fig. 14.4 shows the best fitting models of the combined effects of the ACTN3 and ACE polymorphisms in both male and female subjects. In male subjects, the combined genotypes of the ACTN3 R577X and the ACE I/D polymorphisms are significantly associated with the proportion of type I and type IIx fibers, but not in female subjects. Male subjects with the ACTN3 XX and the ACE ID + DD genotypes exhibit the highest proportion of type I fibers and the lowest proportion of type IIx fibers, whereas male subjects with the ACTN3 RR + RX and the ACE II genotypes exhibit the lowest proportion of type I fibers and the highest proportion of type IIx fibers. Although the combined genotypes of the ACTN3 R577X and the ACE I/D polymorphisms are associated with muscle fiber composition, the contributions are small (5.3% for type I and 7.0% for type IIx fibers). Therefore, further studies are necessary to clarify the role of genetic factors in determining skeletal muscle fiber composition.

14.5 Summary

In this chapter, we focused on genetic polymorphisms such as ACTN3 R577X, ACE I/D, and so on, which are determinants of human muscle fiber composition. It will be necessary to validate associations between these genetic polymorphisms and muscle fiber composition. In addition, functional studies are necessary to support findings. Genetic polymorphisms represent that portion of interindividual phenotypic differences associated with sequence variations in nuclear and mitochondrial DNA sequences. Therefore, genetic variance includes the effects of single genes and gene-environment

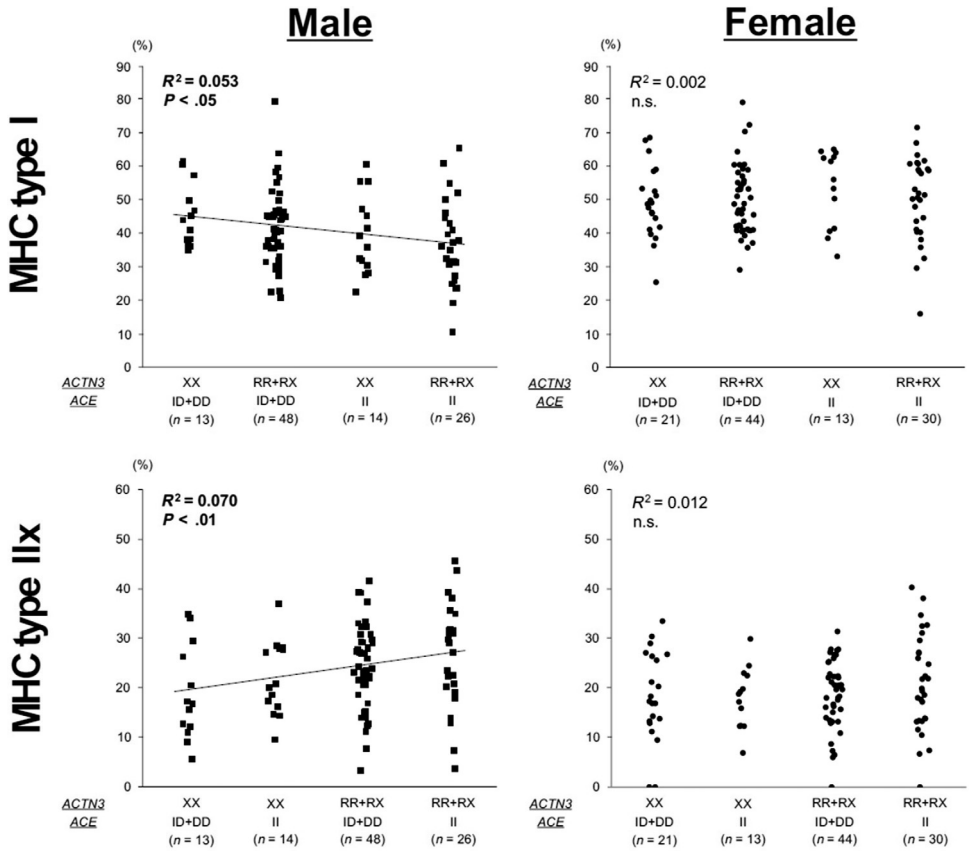

Fig. 14.4 Best-fitting models of the combined effects of *ACTN3* R577X and *ACE* I/D on MyHC-I and MyHC-IIx fibers in men and women.

interactions, as well as gene-gene interactions (i.e., epistasis) (Fuku et al., 2017). Not included within genetic variance are environmental factors, including dietary intake and physical activity levels that effectively predate voluntary nutritional patterns and physical activity, and other lifestyle components that may be influenced by the social and physical environment.

In addition, epigenetic factors may be also important in determining muscle fiber composition. Such environmental factors modulate muscle phenotype via epigenetic mechanisms (methylation/demethylation, acetylation/deacetylation, RNA-mediated processes, and regulation of translation), activation of transcription factors (such as myf5, myoD, MRF4, myogenin, NFATs, PPARδ), and activation of transcriptional coactivators (calcineurin, PGC-1α, PGC-1β). Thus, we need to focus on approaches from several points of view, including -omics approaches (genome, epigenome, transcriptome, proteome, metabolome, and so on) to uncover factors determining muscle fiber composition in the future.

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SECTION IV

Genetics of sport-related diseases and medical conditions

CHAPTER FIFTEEN

Genetics of musculoskeletal soft tissue injuries: Current status, challenges, and future directions

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15.1 Introduction

Regular participation in physical activity is widely accepted as a key component of maintaining a healthy, balanced lifestyle (Reiner et al., 2013; Behrens et al., 2013). However, in spite of the numerous health benefits the risk of sustaining an injury to musculoskeletal soft tissue injuries also increases (Morrow et al., 2012); with over 100 million musculoskeletal soft tissue injuries reported annually worldwide (Ljungqvist et al., 2008). Approximately 30%–50% of these affect tendons and ligaments resulting in acute and chronic sporting injuries such as anterior cruciate ligament (ACL) ruptures, tennis elbow, tendinopathy of the posterior tibialis tendon, Achilles tendinopathy and rotator cuff injuries. Nonetheless, the exact etiology remains to be elucidated though a number of intrinsic and extrinsic risk factors have been implicated in the etiology of these complex, multifactorial conditions (Bahr and Krosshaug, 2005; Posthumus et al., 2011).

In recent years, there has been considerable attention in decoding the genetic basis of musculoskeletal soft tissue injuries, with the aim of identifying the biological pathways and molecular mechanisms involved. In doing so, researchers aspire to use this information to design better therapeutic interventions to assist injured individuals. The high burden of disease and associated costs, together with the negative impact on the quality of life as reported by recreational and elite athletes alike, supports the need for improved pre- and postinjury health care (de Loës et al., 2000). Interestingly, low-to-moderate heritability estimates have been reported for effective responses associated with training and voluntary regular exercise behaviors (Schutte et al., 2017). In addition, the heritability

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estimates for traits associated with athletic performance have been suggested to range between 20% and 80% (Bouchard, 2012; MacArthur and North, 2007; De Moor et al., 2007). Currently, the heritability estimates for musculoskeletal soft tissue injuries are unknown, however, taken into account the heritability of traits linked to both exercise participation and performance, it is not surprising that the evidence for a genetic component to injuries is growing. For example, it was reported by Harvie et al. (2004), that the relative risk of sustaining a full thickness tear of the rotator cuff was significantly higher in siblings compared to spouses of participants with injuries (OR: 2.4, 95% CI: 1.8–3.3). Similarly, in the study by Flynn et al. (2005), individuals with ACL ruptures were twice as likely to have reported a family history of ACL injuries compared to individuals, whom prior to recruitment had not sustained any ACL injuries. Furthermore, Kraemer et al. (2012), using a matched-pair study, reported a positive family history of Achilles tendinopathy as a risk factor for the development of the phenotype (OR: 4.8, 95% CI: 1.1–21.4).

This review, therefore, aims to provide an overview of the current research investigating the genetics of musculoskeletal soft tissue injuries as well as some of the recent advances in the field. Furthermore, some of the current challenges are highlighted and suggestions for future research have been proposed to improve our understanding of the molecular mechanisms underpinning these injuries to inform future treatment and intervention strategies.

15.2 Current status

The majority of studies thus far have employed either familial-based or case-control genetic association studies to investigate how one's genetic profile may contribute to injury susceptibility. However, with the rapid development of modern omics technologies and reducing costs, researchers are now able to apply a hypothesis-free approach to decoding the genetic basis of musculoskeletal soft tissue injuries. Nevertheless, there is still a considerable way to go toward unraveling the genetic basis of musculoskeletal soft tissue injuries. Research thus far has focused on collagenous and noncollagenous matrix components as well as several key cell-signaling factors which collectively modulate cellular homeostasis, and thus contribute to the timely regeneration and adaptation of the tissue in response to mechanical loading. The growing number of implicated genomic regions aid in highlighting the key biological pathways contributing to the pathogenesis of these severe musculoskeletal soft tissue injury phenotypes. To date, more than 80 loci have been associated with the risk of injury, several of which have been implicated in the biological mechanisms predisposing for multiple phenotypes, including those chronic and acute in nature (Rahim et al., 2016a). Many of these multiphenotype associated loci are known to map to genes encoding structural components of the extracellular matrix

(ECM) and other cell-signaling factors. Continued research efforts in this domain will, therefore, enhance our understanding of the underlying genetic, molecular, and cellular mechanisms which can be targeted for use in a clinical setting.

For the purpose of this review, several of the main findings will be presented with a particular focus on associations for which supporting functional evidence is available and variants which have been investigated with several phenotypes and/or in multiple independent cohorts.

15.2.1 Structural components

Type I collagen is the major fibrillar collagen of tendon and ligament structures. The two α1- and one α2-chains of this heterotrimeric molecule are encoded by two genes, *COL1A1* (chr17q21.33) and *COL1A2* (chr7q21.3) respectively. Two common DNA sequence variants, rs1107946 (−1997 G/T) and rs1800012 (+1245 G/T), within the *COL1A1* gene have repeatedly been investigated for their potential association with the risk of musculoskeletal soft tissue injuries (Table 15.1). Interestingly, emerging evidence suggests that there are differences in the genetic profile predisposing for chronic and acute injuries mapping to these loci. Specifically, the rare TT genotype of the Sp1 binding site variant (rs1800012), located within the first intron of the gene, has been associated with decreased risk for acute phenotypes including shoulder dislocations (Khoschnau et al., 2008) ACL ruptures (Posthumus et al., 2009b), and acute soft tissue ruptures in a combined analysis (Collins et al., 2010). In line with this, a recent meta-analysis also reported the association of the rs1800012 TT genotype with reduced risk for sports-related tendon and ligament injuries (Wang et al., 2017). Conversely, the rs1800012 TT genotype was associated with increased risk for intervertebral disk degeneration in the elderly population and increased risk for lumbar disc disease in a cohort of young military recruits (Pluijm et al., 2004; Tilkeridis et al., 2005). Furthermore, the alternate rs1800012 GG genotype was associated with reduced risk for ACL ruptures sustained while skiing (Stępień-Słodkowska et al., 2013); however, the mechanisms resulting in these injuries are proposed to be different to those resulting in ACL ruptures sustained during land-based activities. The rs1107946 variant, located within the promoter region of the *COL1A1* gene, is in linkage disequilibrium with rs1800012 and has been independently associated with the risk of skiing-associated ACL ruptures (Stępień-Słodkowska et al., 2016). Furthermore, a functional haplotype encompassing both variants has been associated with ACL rupture risk in a Polish cohort (Ficek et al., 2013). Both variants have demonstrated the ability to modulate functional properties in vitro (Mann et al., 2001; García-Giralt et al., 2005); however, Jin et al. (2011) hypothesized that it was the interaction of several variants, mapping to the 5′ flank of the *COL1A1* gene that regulated gene function. These interactions were proposed to be

Table 15.1 Genetic polymorphisms within the collagen genes tested for association with musculoskeletal soft tissue injuries

Gene and chromosomal location	Encoded protein	Protein function	Polymorphism	Location	Soft tissue injury	Reference
COL1A1 (17q21)	α1 (I) collagen chain	Major fibrillar collagen	rs1107946 (G/T)	Promoter	ACL[a]	Ficek et al. (2013) and Stępień-Słodkowska et al. (2016)
			rs1800012 (G/T)	Intron 1	ACL[b], shoulder dislocations, acute soft tissue ruptures	Stępień-Słodkowska et al. (2013), Khoschnau et al. (2008), Posthumus et al. (2009a, b), Collins et al. (2010), Ficek et al. (2013), and Wang et al. (2017)
COL3A1 (2q31)	α1 (III) collagen chain	Major fibrillar collagen	rs1800255 (G/A)	Exon 30	ACL	O'Connell et al. (2015) and Stępień-Słodkowska et al. (2014)
COL5A1 (9q34)	α1 (V) collagen chain	Minor fibrillar collagen	rs13946 (C/T)	3′-UTR	LE	Altinisik et al. (2015)
			rs12722 (T/C)		ACL, AT, LE	Mokone et al. (2006), September et al. (2009), Brown et al. (2017), Posthumus et al. (2009a, b), O'Connell et al. (2015), Altinisik et al. (2015), and Lulińska-Kuklik et al. (2018)
			rs3196378 (C/A)		ATP[b]	September et al. (2009) and Brown et al. (2017)
			rs71746744 (−/AGGG) rs16399 (ATCT/−) rs1134170 (A/T)		AT	Abrahams et al. (2013)

COL11A1 (1p21)	α1 (XI) collagen chain	Minor fibrillar collagen	rs3753841 (T/C)	Exon 52	AT[a]	Hay et al. (2013)
			rs1676486 (C/T)	Exon 62		
COL11A2 (6p21)	α2 (XI) collagen chain		rs1799907 (T/A)	Intron 6	AT[a]	Hay et al. (2013)
		FACIT collagen	rs970547 (A/G)	Exon 65	ACL	Posthumus et al. (2010) and O'Connell et al. (2015)
COL27A1 (9q32)	α1 (XXVII) collagen chain	Minor fibrillar collagen	rs946053 (G/T)	Intron 41	AT[a]	Saunders et al. (2013)

Abbreviations: *ACL*, anterior cruciate ligament ruptures; *AT*, Achilles tendinopathy; *ATP*, Achilles tendon pathology; *FACIT*, fibril-associated collagens with interrupted triple helices; *LE*, lateral epicondylitis (tennis elbow); *PTT*, tendinopathy of the posterior tibialis tendon.
[a] Indicates the variant was associated as part of an inferred haplotype or an inferred allele combination.
[b] Indicates the variant was independently associated with the musculoskeletal soft tissue injury, as well as part of an inferred haplotype or an inferred allele combination.

regulated by chromatin looping (Jin et al., 2011). In contrast to these associations, several studies have failed to associate these variants with the risk of musculoskeletal soft tissue injuries (Posthumus et al., 2009b; Erduran et al., 2014). This may potentially indicate that several independent studies have been insufficiently powered to detect the rare rs1800012 TT genotype and may explain the conflicting results when comparing the data from the larger combined-analysis to that of smaller independent cohorts.

Type V collagen is a minor fibrillar collagen involved in the modulation of collagen fibril diameter and fibril assembly and is, therefore, an important structural component of tendons and ligaments (Birk et al., 1990). Several studies have focused on the *COL5A1* gene (chr9q34.3) which encodes the α1(V) chain of this heterotrimeric molecule. Specifically, researchers have investigated a number of variants located within the 3′-untranslated region (UTR) of the *COL5A1* gene including rs13946 (T/C), rs14776422 (C/T), rs5574880 (G/A), rs12722 (T/C), rs3196378 (C/A), rs71746744 (−/AGGG), rs16399 (−/ATCT), and rs1134170 (A/T). Several independent and haplotype associations were reported with Achilles tendinopathy (Mokone et al., 2006; September et al., 2009; Brown et al., 2017), Achilles tendon ruptures (Brown et al., 2017), tennis elbow (Altinisik et al., 2015), and ACL ruptures in females (Posthumus et al., 2009a; O'Connell et al., 2015; Lulińska-Kuklik et al., 2018) (Table 15.1).

As the 3′-UTR of genes usually contains important posttranscriptional regulatory elements Laguette et al. (2011) and Abrahams et al. (2013) investigated the functionality of the *COL5A1* 3′-UTR (Abrahams et al., 2013; Laguette et al., 2011). The authors identified two major allelic forms of the *COL5A1* 3′-UTR consisting of a set of seven strongly linked sequence variants and found these variants affect *COL5A1* mRNA secondary structure (Abrahams et al., 2013; Laguette et al., 2011). The "T functional form" was generally identified in Achilles tendinopathic patients and demonstrated increased mRNA stability compared to the "C functional form" which corresponds to the wild-type sequence (Laguette et al., 2011). Abrahams et al. (2013) also observed the microRNA, Hsa-miR-608 (*MIR608*) putatively binds to the 3′-UTR and regulates *COL5A1* mRNA stability. Furthermore, investigation of the functional *MIR608* rs4919510 (C/G) variant identified the CC genotype to be associated with increased Achilles tendinopathy risk (Abrahams et al., 2013) (Table 15.2). Collectively, this data formed the basis of a novel functional hypothesis for the role of the *COL5A1* 3′-UTR in injury risk. Collins and Posthumus (2011) proposed that altered *COL5A1* mRNA stability may alter type V collagen production with potential implications on collagen fibril architecture and structure and, consequently, the biomechanical properties of the tissue (Collins and Posthumus, 2011). Alongside the above-mentioned genes, associations have also been noted for the *COL3A1* (O'Connell et al., 2015; Stępień-Słodkowska et al., 2014), *COL11A1* (Hay et al., 2013), *COL11A2* (Hay et al., 2013), *COL12A1* (Posthumus et al., 2010; O'Connell et al., 2015), and *COL27A1* genes (Saunders et al., 2013) (Table 15.1).

Table 15.2 Genetic polymorphisms within genes encoding regulatory components of the extracellular matrix tested for association with musculoskeletal soft tissue injuries

Gene and chromosomal location	Encoded protein	Protein function	Polymorphism	Location	Soft tissue injury	Reference
TNC (9q33)	Tenascin-C glycoprotein	Regulates cell-matrix interactions	rs1061494 (C/T)	Exon 4	AT[b]	Gibbon et al. (2018)
			rs1330363 (G/A)	Intron 15	AT[a]	Saunders et al. (2013)
			rs2104772 (T/A)	Exon 17	AT[a], ACL	Saunders et al. (2013) and Gibbon et al. (2018)
			G-T tandem repeat	Intron 17	AT	Mokone et al. (2005)
			rs13321 (G/C)	Exon 24	AT[a]	Saunders et al. (2013)
			rs1138545 (G/A)	Exon 10	RCT[b]	Kluger et al. (2017a, b)
			rs3789870 (C/T)	Intron 10		
			rs7021589 (A/G)	Intron 17		
			rs10759753 (T/C)	Intron 19		
			rs72758637 (G/C)	Intron 19		
			rs7035322 (G/T)	Intron 28		
FBN2 (5q23-q31)	Fibrillin-2	Component of connective tissue microfibrils	rs331079 (G/T)	Intron 7	ACL, AT	El Khoury et al. (2014)
MIR608 (10q24)	microRNA 608	Regulation of *COL5A1* mRNA stability	rs4919510 (C/G)	3′-UTR	ATP	Abrahams et al. (2013) and Brown et al. (2017)

Continued

Table 15.2 Genetic polymorphisms within genes encoding regulatory components of the extracellular matrix tested for association with musculoskeletal soft tissue injuries—cont'd

Gene and chromosomal location	Encoded protein	Protein function	Polymorphism	Location	Soft tissue injury	Reference
ACAN (15q26)	Aggrecan	Major proteoglycan of articular cartilage—contributes to load-bearing properties of cartilage	rs2351491 (C/T)	Exon 11	ACL[a]	Mannion et al. (2014) and Cięszczyk et al. (2017)
			rs1042631 (C/T)			
			rs1516797 (T/G)	Intron 12	ACL[b]	
BGN (Xq28)	Biglycan	Involved in collagen fibrillogenesis	rs1126499 (C/T)	Exon 4	ACL[a]	Mannion et al. (2014), Cięszczyk et al. (2017), and Willard et al. (2018)
			rs1042103 (G/A)	Exon 8		
DCN (12q21)	Decorin		rs13312816 (C/T)	Intron 1	ACL[a]	Mannion et al. (2014)
			rs516115 (T/G)	Intron 3	ACL[b]	
LUM (12q21)	Lumican		rs2268578 (T/C)	Intron 3	ACL[a]	Mannion et al. (2014)

Abbreviations: *ACL*, anterior cruciate ligament ruptures; *AT*, Achilles tendinopathy; *ATP*, Achilles tendon pathology; *RCT*, rotator cuff tendinopathy.
[a] Indicates the variant was associated as part of an inferred haplotype or an inferred allele combination.
[b] Indicates the variant was independently associated with the musculoskeletal soft tissue injury, as well as part of an inferred haplotype or an inferred allele combination.

15.2.2 Extracellular matrix regulatory components

The gene encoding the tenascin-C glycoprotein (*TNC*) is encoded on chromosome 9 (chr9q33.1). Tenascin-C is an adhesion-modulating protein, regulating the proliferation, migration, and adhesion of certain cell types and other ECM components within the cellular environment (Chiquet-Ehrismann and Chiquet, 2003). Mokone et al. (2005) first implicated a guanine-thymine (GT) dinucleotide repeat polymorphism with the risk of chronic AT (Mokone et al., 2005) (Table 15.2). Subsequently, two other *TNC* variants, rs1330363 (A/G) and rs2104772 (A/T) were associated with AT risk (Saunders et al., 2013). This latter study also demonstrated the association of an inferred haplotype (G-C-A) spanning the *COL27A1* gene (rs946053 G/T) together with two of the investigated *TNC* variants (rs13321 G/C and rs2104772 A/T).

Kluger et al. (2017a) identified several variants associated with the risk of rotator cuff tears (RCT). The association of the nonsynonymous rs1138545 (C/T) variant was highlighted as potentially, biologically relevant. This variant is known to alter the amino acid sequencing close to the bridging sequencing between two adjacent fibronectin type III repeat domains within the mature tenascin-C peptide. In addition to rs1138545, the rs2104772 variant, previously associated with Achilles tendinopathy was also associated with RCT (Kluger et al., 2017a) Proceeding this study, Kluger et al. (2017b) identified a *TNC* inferred haplotype (C-A-G; rs1138545 C/T-rs2104772 A/T-rs10759752 A/G) associated with the risk of large recurrent defects post RCT corrective surgery (Kluger et al., 2017b). In contrast to these results, Lulińska-Kuklik et al. (2019a) reported the lack of any significant associations with the risk of ACL ruptures and three previously investigated *TNC* variants (rs1330363 C/T, rs2104772 A/T, and rs13321 G/C respectively). Interestingly, however, the rs2104772 (A/T) variant was associated with capillary remodeling following endurance exercise. Specifically, carriers of the rs2104772 AA genotype demonstrated a significant increase in capillary density within skeletal muscles compared to carriers of the alternate T allele (Valdivieso et al., 2017). However, the sample size was small, and therefore additional studies are required to validate these results. In addition to *TNC*, several studies have investigated genes encoding a number of proteoglycans with ACL rupture risk in independent cohorts from South Africa (Mannion et al., 2014; Willard et al., 2018) and Poland (Cięszczyk et al., 2017) (Table 15.2).

The *MMP3* gene is one of nine *MMP* genes clustered on the long arm of chromosome 11 (chr11q22). The encoded zinc-dependent endopeptidase (stromelysin 1) degrades several structural and modular proteins; thereby contributing to connective tissue matrix remodeling. Raleigh et al. (2009) first demonstrated the association of three single nucleotide variants within *MMP3*:rs679620 (A/G), rs591058 (T/C) and rs650108 (G/A), with the risk of chronic Achilles tendinopathy (Raleigh et al., 2009) (Table 15.3). Specifically, the rs679620 GG genotype, rs591058 CC genotype, and rs650108 AA genotype were associated with increased injury risk (Raleigh et al., 2009). Furthermore, the A-T-G

Table 15.3 Genetic polymorphisms within genes encoding metalloproteinases and inhibitors of metalloproteinases tested for association with musculoskeletal soft tissue injuries

Gene and chromosomal location	Encoded protein	Protein function	Polymorphism	Location	Soft tissue injury	Reference
MMP1 (11q22)	Matrix metalloproteinase 1	Degradation of collagenous and non-collagenous extracellular matrix components	rs1799750 (1G/2G)	Promoter	ACL[a], PTT[b], RCT	Posthumus et al. (2012), Baroneza et al. (2014), Godoy-Santos et al. (2013), and Assunção et al. (2017)
			rs1144393 (A/G)		PTT[b]	Baroneza et al. (2014)
MMP3 (11q22)	Matrix metalloproteinase 3		rs3025058 (5A/6A)	Promoter	ACL, AT[b]	Malila et al. (2011) and Gibbon et al. (2017)
			rs679620 (A/G)	Exon 2	ACL[a], ATP[b], hamstring injury	Posthumus et al. (2012), Raleigh et al. (2009), Gibbon et al. (2017), El Khoury et al. (2016); Larruskain et al. (2018), and Lulińska-Kuklik et al. (2019a, b)
			rs591058 (T/C)	Intron 4	ATP[b]	Raleigh et al. (2009), Gibbon et al. (2017), and Lulińska-Kuklik et al. (2019a, b)
			rs650108 (A/G)	Intron 8		

MMP8 (11q22)	Matrix metalloproteinase 8		rs11225395 (C/T)	Promoter	PTT	Godoy-Santos et al. (2014)
MMP10 (11q22)	Matrix metalloproteinase 10		rs486055 (C/T)	Exon 1	ACL[a]	Posthumus et al. (2012)
MMP12 (11q22)	Matrix metalloproteinase 12		rs2276109 (A/G)	Promoter	ACL[b]	Posthumus et al. (2012)
TIMP2 (17q25)	Metalloproteinase inhibitor 2	Essential regulator of ECM turnover and remodeling	rs4789932 (C/T)	Promoter	ATP	El Khoury et al. (2013, 2016)

Abbreviations: *ACL*, anterior cruciate ligament ruptures; *AT*, Achilles tendinopathy; *ATP*, Achilles tendon pathology; *ECM*, extracellular matrix; *PTT*, tendinopathy of the posterior tibialis tendon; *RCT*, rotator cuff tendinopathy.
[a] Indicates the variant was associated as part of an inferred haplotype or an inferred allele combination.
[b] Indicates the variant was independently associated with the musculoskeletal soft tissue injury, as well as part of an inferred haplotype or an inferred allele combination.

inferred haplotype (rs679620-rs591058-rs650108) was associated with decreased risk for chronic Achilles tendinopathy in the same study (Raleigh et al., 2009). The rs679620 variant was also associated with acute Achilles tendon ruptures in a British cohort; however, only a small number of cases were investigated (El Khoury et al., 2016). More recently, the rs679620 G and the rs591058 C alleles were associated with increased risk for noncontact ACL rupture risk in a Polish cohort of football players (Lulińska-Kuklik et al., 2019b).

Posthumus et al. (2011) proposed that the *MMP3* rs679620 variant may interact with several other MMP loci, *MMP10* rs485055 (C > T), *MMP1* rs1799750 (1G > 2G), and *MMP12* rs2276109 (A > G), to modify susceptibility to ACL ruptures in a South African cohort (Posthumus et al., 2012) (Table 15.3). Furthermore, the functional promoter variant, rs3025058, which is tagged by rs679620 was independently associated with ACL ruptures sustained through contact mechanisms in an independent Thai population (Malila et al., 2011) and was inferred to be associated with risk of Achilles tendinopathy in the study by Gibbon et al. (2017). Interestingly, rs679620 was the only variant of 37 to associate with the risk of acute, severe, and recurrent hamstring injuries in a cohort of male soccer players of Spanish ancestry (Larruskain et al., 2018). In accordance, rs679620 was also associated with postoperative stiffness, consequently decreasing range of motion after RCT in a Chinese Hans cohort. Conversely to these findings, other studies have failed to implicate these *MMP3* variants as modifiers of risk for musculoskeletal soft tissue injuries or with certain biomechanical properties of connective tissues (Foster et al., 2014; Burger et al., 2016). Therefore, the inconsistencies in results may suggest that either more informative markers of risk mapping to the 11q22 chromosomal region may still require identification or that there may be important differences in the genetic signatures predisposing for different injury phenotypes mapping to the *MMP* genes.

15.2.3 Signaling factors

The ECM of connective tissues is highly dynamic and continuously undergoes remodeling in order to maintain homeostasis. This process is tightly regulated by numerous cytokines, growth factors and signaling molecules—all regulating the degradation and production of new structural components (Yang et al., 2005; Jiang et al., 2012). It is necessary to consider that the matrix remodeling pathway is highly complex and intricate and therefore it is likely that several compensatory mechanisms may be in place in an attempt to maintain balance. Nevertheless, dysregulation of this pathway can occur and is hypothesized to increase susceptibility to injury (Bonnans et al., 2014).

Interleukins are a group of cytokines that are able to modulate cellular behavior and play key roles in cell signaling. Several studies have investigated genes encoding several interleukin proteins including interleukin-1β (*IL1B* rs16944 C/T), interleukin-1 receptor antagonist [*IL1RN* rs2234663 variable nucleotide tandem repeat (VNTR)],

interleukin-6 (*IL6* rs1800795 G/C), and interleukin-6 receptor (*IL6R* rs2228145 A/C) with risk of Achilles tendinopathy and ACL ruptures in cohorts from South Africa, Australia, and the United Kingdom (September et al., 2011; Brown et al., 2017; Rahim et al., 2017) (Table 15.4). Independent associations were noted for the *IL1B* rs16944 (C/T) promoter polymorphism and increased risk of both Achilles tendon ruptures (Brown et al., 2017) and ACL ruptures (Rahim et al., 2017), suggesting a possible predisposition for acute injuries. Although no independent associations were noted with the risk of Achilles tendinopathy, collectively the *IL1B*, *IL1RN*, and *IL6* variants were observed to modulate the risk of tendinopathy in combination with the previously associated *COL5A1* rs12722 variant (September et al., 2011). Moreover, a similar inferred allele combination (*IL1B*, *IL6*, *IL6R*, and *COL5A1*) was associated with risk of ACL ruptures (Rahim et al., 2017). Interestingly, the associations only differed at the alleles implicated for the *IL1RN* rs2234663 and *IL6* rs1800795 loci. Collectively, these results potentially implicate interleukin signaling cascades in the underlying biological mechanisms predisposing for both acute and chronic musculoskeletal soft tissue injuries. Though unsurprising, given that interleukin-1β and interleukin-6 are responsible for activating many downstream signaling cascades (Tsuzaki et al., 2003; Legerlotz et al., 2012), further research is required to elucidate their roles in musculoskeletal soft tissue injury pathophysiology.

The angiogenesis-signaling cascade is a component of the more complex matrix remodeling pathway and is regulated by a myriad of growth factors, cytokines, and signaling factors (Petersen et al., 2003, 2004; Egginton, 2009) as well as extrinsic factors such as mechanical loading (Petersen et al., 2004; Egginton, 2009; Mousavizadeh et al., 2014). Tendinopathic tissues are often characterized by increased neovascularization; as has been noted in degenerative Achilles tendons (Aström and Rausing, 1995; Pufe et al., 2001), rotator cuff tendinopathy (Lakemeier et al., 2010; Lewis et al., 2009) as well as in ruptured tendons (Pufe et al., 2001) and ligaments (Beye et al., 2008). Vascular endothelial growth factor (VEGF) is instrumental to the angiogenesis signaling cascade with protein expression influenced by genetic variation in the *VEGFA* gene (chr6p21.1) (Brogan et al., 1999; Shahbazi et al., 2002).

Several independent and haplotype associations were reported for the *VEGFA* (rs699947 C/A, *VEGFA* rs1570360 G/A, and *VEGFA* rs2010963 G/C) gene with risk of musculoskeletal soft tissue injuries (Rahim et al., 2014, 2016b, 2018) (Table 15.4). Specifically, the *VEGFA* rs699947 CC was associated with an increased risk of noncontact ACL ruptures (Rahim et al., 2014) but in contrast the same genotype was associated with reduced risk of Achilles tendinopathy (Rahim et al., 2016b) A similar pattern was observed when analyzing the inferred haplotype combinations. The C-G-C allelic combination, associated with increased VEGF production (Lambrechts et al., 2003), conferred an increased risk of ACL rupture whereas the low-VEGF producing haplotype (A-A-G) (Lambrechts et al., 2003) was associated with a reduced risk of injury (Rahim

Table 15.4 Genetic polymorphisms within genes encoding cell signaling molecules tested for association with musculoskeletal soft tissue injuries

Gene and chromosomal location	Encoded protein	Protein function	Polymorphism	Location	Soft tissue injury	Reference
IL-1B (2q14)	Interleukin-1β	Role in the inflammatory pathway and ECM degradation	rs16944 (T/C)	Promoter	ACL[b], AT[a], RUP	September et al. (2011), Rahim et al. (2017), and Brown et al. (2017)
			rs1143627 (C/T)			
IL-1RN (2q14)	Interleukin-1 receptor antagonist	Antagonist for IL-1α and IL-1β	rs2234663 VNTR	Intron 2	ACL[a], AT[a]	September et al. (2011)
IL-6 (1q21)	Interleukin-6	Role in apoptosis and the inflammatory pathway	rs1800795 (G/C)	Promoter	ACL[a], AT[a]	September et al. (2011) and Rahim et al. (2017)
IL-6R (1q21)	Interleukin-6 receptor	Receptor for IL-6	rs2228145 (A/C)	Exon 9	ACL[a]	Rahim et al. (2017)
VEGFA (6p21)	VEGF-A isoform	Essential regulator of angiogenesis	rs699947 (C/A)	Promoter	ACL[b], ATP[b]	Rahim et al. (2016a, b) and Rahim et al. (2014)
			rs1570360 (G/A)	Promoter		
			rs2010963 (G/C)	3'-UTR		
KDR (4q11-4q12)	Kinase insert-domain receptor	Receptor for VEGF and mediates VEGF signaling	rs2071559 (G/A)	Promoter	ACL[b], AT[a]	Salles et al. (2016), Rahim et al. (2014), Rahim et al. (2016a, b), and Rahim et al. (2018)
			rs2305948 (G/A)	Exon 7	AT[b]	
			rs1870377 (T/A)	Exon 11	ACL[b], AT[a]	
GDF5 (20q11)	Growth differentiation factor 5	Regulates cell growth and differentiation	rs143383 (T/C)	5′-UTR	ATP	Posthumus et al. (2010)
CASP8 (2q33-q34)	Caspase 8	Initiator caspase	rs3834129 (ins/del)	Promoter	ACL[b], AT[b]	Nell et al. (2012), Brown et al. (2017), and Rahim et al. (2017)
			rs1045485 (G/C)	Exon 9		

Abbreviations: *ACL*, anterior cruciate ligament ruptures; *AT*, Achilles tendinopathy; *ATP*, Achilles tendon pathology; *ECM*, extracellular matrix; *RUP*, Achilles tendon ruptures; *VEGF*, vascular endothelial growth factor.

[a]Indicates the variant was associated as part of an inferred haplotype or an inferred allele combination.

[b]Indicates the variant was independently associated with the musculoskeletal soft tissue injury, as well as part of an inferred haplotype or an inferred allele combination.

et al., 2014). Alternatively, the low-VEGF producing haplotype (A-G-G) was also associated with increased risk of tendinopathy (Rahim et al., 2016b). Interestingly, a recent study by Wezenbeek et al. (2018b) documented that the lower the increase in blood flow after running (Wezenbeek et al., 2018b), the higher the risk for developing AT and this effect was observed to be age and sex-dependent (Wezenbeek et al., 2018a).

The biological effects of VEGF are mediated via its receptor kinase insert-domain receptor (KDR). Inferred haplotype analysis implicated the *KDR* gene (chr4q12) with ACL rupture risk. Specifically, the *KDR* (rs2071559 A/G, rs1870377 A/T) G-A inferred haplotype was significantly associated with increased risk of ACL ruptures (Rahim et al., 2014). The rs2071559 G allele is hypothesized to alter a transcription factor binding site in the promoter region thus reducing *KDR* transcription (Wang et al., 2007) whilst the A allele of the nonsynonymous rs1870377 T/A variant was previously associated with reduced VEGF-binding efficacy to KDR (Wang et al., 2007). Therefore, these haplotype associations may be defining a region within KDR to be involved in regulating the binding affinity of VEGF. No independent or haplotype associations were noted for *KDR* and Achilles tendinopathy risk in a study group from South Africa (Rahim et al., 2016b) though the G-A inferred haplotype was associated with reduced Achilles tendinopathy risk in a group of volleyball players from Brazil (Salles et al., 2016) (Table 15.4).

Furthermore, the same five *VEGFA* and *KDR* variants were investigated with the risk of ACL ruptures in a South African (SA) indigenous cohort of mixed ancestry (Rahim et al., 2018). No independent or haplotype associations were observed for *VEGFA* though similar genotype and haplotype associations were observed for the *KDR* gene as compared to a South African White cohort (Rahim et al., 2014). As a result, it may be hypothesized that there are population-specific markers for ACL rupture risk and although the selected *VEGFA* variants were informative in a self-identified White cohort, they were not informative in the SA mixed ancestry cohort. This may potentially be due to the greater heterogeneity observed in the indigenous SA population resulting from differences in genetic ancestry (Patterson et al., 2010; de Wit et al., 2010; Greeff, 2007; Geldenhuys et al., 2014).

The reasons for the dissimilar associations between ACL ruptures and Achilles tendinopathy are not fully understood. These may be due to the differences between the two injury models (acute vs chronic) or as a result of the functional and molecular differences between ligament and tendon tissues. Previous studies have also reported similarities and differences in genetic associations between the two injury models (Collins et al., 2015; September et al., 2012). Though ligaments and tendons are structurally and compositionally similar, they differ in their functional capacities and therefore the biological impact of the differences observed at a genetic level may have implications on the development of acute and chronic injuries. Another explanation is that the loci are in linkage with a true risk-conferring variant indicating a more comprehensive examination of the genomic interval is necessary. Moreover, the associations need to be

investigated at a functional level to assess the biological significance of these variants in both acute and chronic injuries.

15.3 Hypothesis-free approach

The majority of studies investigating the genetic basis of musculoskeletal soft tissue injuries have followed a candidate gene, case-control genetic association approach. This approach is hypothesis-driven whereby the candidate gene and prioritized variants are selected based on an a priori hypothesis that the gene product is involved in the biological mechanisms underpinning injury development. However, due to the paucity in knowledge regarding these biological mechanisms, only a select number of genes have been prioritized and only variants previously associated with injury risk have been further investigated in other independent populations. Therefore, the ability to identify all risk-conferring variants for musculoskeletal soft tissue injuries using this approach alone is limited. The omics era is well established and the use of microarray technology and high throughput sequencing technologies to explore the genome is effectively being exploited to further define the genetic risk profile of complex, multifactorial phenotypes including musculoskeletal soft tissue injuries.

Baird et al. (2014), using a genome-wide association study (GWAS), identified regions of the canine chromosomes 1, 3, and 33 associated with the risk of cranial cruciate ligament ruptures (CCLR). Specifically, the genes encoding the sortilin-related VPS10 domain containing receptor 2 (*SORCS2*) and semaphorin 5B (*SEMA5B*) were implicated in susceptibility for CCLR. Interestingly, both these genes have human orthologs, suggesting the potential involvement of neurological signaling pathways in the modulation of ACL injuries. The GWAS conducted by Baker et al. (2017) identified an additional 99 loci associated with the risk of ACL ruptures (Baker et al., 2017); however, although both studies used DNA obtained from domestic dog breeds, there was little overlap in the associated genetic loci.

A GWAS in humans identified two variants associated with the risk of RCT. Specifically, the rs820218 (G/A) and rs12527089 (C/T) variants in the genes encoding the SAP30-binding protein (SAP30BP) and the sterile motifs-and SH3 domain containing protein-1 (SASH1) were associated with injury risk (Tashjian et al., 2016). Both genes function within the cellular process of apoptosis. Conversely, Kim et al. (2017) were unable to identify any variants associated with the risk of either AT or ACL ruptures using a false discovery rate of less than 5% (Kim et al., 2017). Although these GWASs have successfully identified variants associated with the risk of injury, these microarray-based genotyping platforms are limited in their resolution, meaning that proportions of the genome are not directly genotyped. However, the advancement of next-generation sequencing has significantly lowered the cost of sequencing, thereby providing a molecular tool for the interrogation of the genome at a base-pair resolution.

To date, no study has utilized whole genome sequencing (WGS) to investigate the genetic basis of musculoskeletal soft tissue injuries. This may potentially reflect the high cost associated with this approach and the fact that processing the data is a complex task, often requiring the assistance of an experienced bioinformatician. Furthermore, much of the genome remains uncharacterized and; therefore, exhausting financial resources to obtain a massive amount of data not immediately transferable to the phenotype under investigation has warranted consideration by researchers. Currently, there are no WGS datasets specific to musculoskeletal soft tissue injuries in the public domain.

A cost-effective alternative to WGS is targeted sequencing of the genome including whole exome sequencing (WES). To date, only two studies have been published demonstrating the use of WES to identify genetic loci associated with musculoskeletal soft tissue injury risk. Firstly, Caso et al. (2016) conducted a family-based study whereby a pair of fraternal twins, both of whom had suffered spontaneous noncontact ACL ruptures, were sequenced along with several other family members (Caso et al., 2016). Although several of the 11 highlighted loci are plausible candidates based on their biological function or location, none have previously been prioritized for interrogation using hypothesis-driven approaches (Caso et al., 2016).

Alternatively, a recent study adopted a hybrid approach in which selected participants representing divergent extremes of the phenotype spectrum for Achilles tendinopathy were sequenced using WES, but the initial analysis of the generated data involved targeting previously implicate genomic regions (Gibbon et al., 2018). Using a customized tiered filtering strategy involving a predetermined allele frequency difference threshold, new candidate-variants within previously implicated genes were prioritized for further interrogation in larger independent cohorts. The *TNC* gene was prioritized for interrogation using this approach. In total four variants were prioritized for genotyping from the WES generated dataset, of which the rs1061494 variant was associated with chronic Achilles tendinopathy. Furthermore, an inferred haplotype (T-T; rs1061494 C/T-rs2104772 A/T) spanning two of the variants prioritized from the WES dataset, was associated with reduced Achilles tendinopathy risk (Gibbon et al., 2018).

Collectively, the evidence from candidate gene and high-throughput studies add to our understanding of the genetic predisposition to musculoskeletal soft tissue injuries thereby allowing researchers to infer some of the biologically significant pathways involved in pathophysiology. However, substantially more research is required in this domain before these results can be targeted for therapeutic interventions or be of clinical significance.

15.4 Future directions

Some of the main limitations to the studies conducted thus far are the small sample sizes and limited functional experiments to support the reported genetic associations

(Jin et al., 2009; Laguette et al., 2011). Furthermore, the majority of the reported associations has been conducted in White sample groups and is yet to be replicated in other ancestral groups. Accordingly, further research in large, independent cohorts is required. Researchers should also be aware of the confounding effects of sex and population stratification and therefore consider examining sex-specific and population-specific markers as these may be more informative.

It is apparent from the current literature that integrated approaches are required to further facilitate the interrogation of the genome to refine the biological signatures underpinning musculoskeletal soft tissue injury susceptibility. Therefore, it is imperative that large-scale consortiums are established, containing data of well-phenotyped individuals. This may prove particularly beneficial for forthcoming sequencing studies to detect rare polymorphisms with larger effect sizes (Pitsiladis et al., 2016). In addition, the functionality of the reported associations requires investigation. By considering tissue-specific effects on matrix remodeling in response to mechanical loading, we may gain insight into the effect of genetic variation on tendon and ligament capacity and thereby how they may modulate injury risk. Another area of research which requires exploration is the macrophage response and its involvement in matrix remodeling, repair, and healing (Lieberthal et al., 2015; Wu et al., 2014). The establishment of international consortia is, therefore, an essential objective toward a multidisciplinary approach to unravel the pathobiology of musculoskeletal soft tissue injuries. It is only through collaboration and sharing of data and resources that we can begin to build upon preexisting knowledge regarding clinically relevant risk-conferring loci for these debilitating injuries.

15.5 Conclusion

Although the exact underlying pathobiology of musculoskeletal soft tissue injuries is not clearly understood, there is an increasing amount of empirical evidence supporting the genetic predisposition to injury risk. With the aid of genetic association studies, researchers can begin to identify potentially significant pathways underpinning risk and recovery that can be targeted for therapeutic interventions. Collaboratively, this information can be used by clinicians, coaches, and athletes to develop personalized programs for prehabilitation of susceptible individuals or to aid recovery after overloading/injury. Currently, genetic testing has limited potential as a diagnostic or prognostic tool for multifactorial injuries (Webborn et al., 2015) and so for this data to have meaningful clinical application, intrinsic and extrinsic risk factors will need to be considered collectively (Meeuwisse, 1994; Collins et al., 2018).

Research thus far has highlighted several key candidates requiring further interrogation at the genomic and functional levels whilst modern omics and sequencing technologies have the potential to identify novel candidates underpinning injury susceptibility. In order to achieve these goals and aspire toward clinically relevant biological targets, it is imperative

that international consortia are established through which we will be able to collect and evaluate large, well-phenotyped data sets by uniting resources and knowledge.

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CHAPTER SIXTEEN

Genetics of sport-related concussion

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16.1 Introduction

A concussion is a common head injury in sports, with an estimated 1.6–3.8 million sports-related concussions occurring annually in the United States of America (Langlois et al., 2006). A concussion is defined as a traumatic brain injury (TBI) induced by biomechanical forces, which causes the rapid onset of short-lived neurological impairment and a range of clinical and cognitive signs and symptoms that resolve spontaneously with time (McCrory et al., 2017). Concussion may be caused by either a direct impact to the head or neck or through a blow to the body that causes forces to be transmitted to the head (McCrory et al., 2017). Although concussion was classified as a subset of TBI (McCrory et al., 2017), the distinction is controversial due to the differences between the injury constructs (Voss et al., 2015; McCrory et al., 2013). On a TBI severity scale, concussion injuries are viewed as being on the less severe side of mild traumatic brain injuries (mTBI's) (Teasdale and Jennett, 1974). However, the concussion has at times been used interchangeably with mTBI in the scientific literature.

A concussion is further defined as a functional rather than structural injury (McCrory et al., 2017). Although neuropathological changes may be evident, no physical abnormality is visible on standard structural neuroimaging modalities following concussion (Shackford et al., 1992; McCrory et al., 2017). The symptoms experienced are the consequence of a complex sequence of molecular and neurotransmission changes that cause transient disturbances in brain function. The molecular pathophysiology of concussion includes excessive neurotransmitter release and depolarization, calcium influx, mitochondrial dysfunction, decreased energy production, neuroinflammation, and apoptosis in the affected area (Giza and Hovda, 2014). These cellular level changes result in the broad spectrum of signs and symptoms observed following a concussion. These difficulties may include somatic symptoms (e.g., headache), cognitive problems (e.g., difficulty concentrating), emotional changes (e.g., lability), physical signs (e.g., loss of consciousness), balance impairments (e.g., gait unsteadiness), behavioral changes (e.g., irritability), and sleep/wake disturbances (e.g., drowsiness) (McCrory et al., 2017).

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Concussion symptoms are usually most severe in the first 48 hours (Barth et al., 1989) and then gradually resolve in the following days, weeks, or months (McClincy et al., 2006; Ryan and Warden, 2003). Being diagnosed with a concussion can thus lead to significant absence from school, work, and sporting participation. For some, this may result in diminished academic or professional progress, loss of income, psychological stress, or early retirement from sport (Ellis et al., 2016; Covassin et al., 2014; Ransom et al., 2015). In addition, these concussion injuries can cause severe detrimental consequences (Manley et al., 2017; Mckee et al., 2009; Baugh et al., 2012). For example, the controversial and rare second impact syndrome, in which an athlete still recovering from a concussion sustains a subsequent concussion, leading to catastrophic brain swelling and death (Cantu, 1998). However, it must be noted that there have been less than 20 reported cases of second impact syndrome in the literature and the understanding of these events are largely based on anecdotal evidence (Dessy et al., 2015).

More recently, and perhaps more pertinently, several scientific reports have raised the concern that a history of multiple concussions and sub-concussive impacts may lead to reduced neurological functioning later in life (Gardner et al., 2010; Dupuis et al., 2000; De Beaumont et al., 2007). Most notable is chronic traumatic encephalopathy (CTE), which is defined as "a progressive neurodegenerative syndrome caused by single or usually repetitive blunt force impacts to the head" (Bailes et al., 2015). In sport, CTE is associated with repetitive concussive events and prolonged exposure to head impacts in athletes (Stein et al., 2015). CTE is described as a neurodegenerative tauopathy that has a predictable pattern of hyperphosphorylated tau deposits and widespread axonal damage in the brain (Kulbe and Hall, 2017; Mckee et al., 2013; Mez et al., 2017). Symptoms of CTE include progressive and dramatic cognitive, behavioral, and mood changes (Mckee et al., 2013). To date, 153 former athletes and military veterans have been diagnosed with CTE. Despite recent advances into the neuropathology of CTE, very little is known about the syndrome and the exact relationship between CTE and concussion (Montenigro et al., 2015).

Given the health and economic burden of these injuries (Thurman, 2001; Manley et al., 2017; Mckee et al., 2009; Baugh et al., 2012), identifying the underlying risk factors for concussion is imperative to (i) identify those at increased susceptibility to concussion injuries and poor recovery trajectories, (ii) provide further information on the underlying pathophysiology of concussion, and (iii) improve prevention, management, and treatment strategies. Like most sports injuries, concussion is regarded as a multifactorial disorder affected by both intrinsic and extrinsic factors. Intrinsic factors are those that come from within the body, while extrinsic factors emanate from outside of the body. The documented individual variability in concussion susceptibility and outcomes (Kutcher and Eckner, 2010; Mccrea et al., 2014; Cancelliere et al., 2014) suggests a strong intrinsic and possibly genetic influence (McDevitt et al., 2014; Mccrea et al., 2017; Abrahams et al., 2014).

There has been considerable amount of genetic research in TBI (Dardiotis et al., 2010), but in recent years, this investigation has extended into the field of sport-related concussion. Specifically, studies have aimed to investigate whether there is a specific genetic profile that may lead to (i) increased concussion susceptibility or (ii) poor concussion outcomes. Although the field of concussion genetics is still relatively in its infancy, it is predicted to have major translational significance to the evidence- based approach to the clinical management of concussion in the future (Mccrea et al., 2017).

16.2 The genetics of concussion susceptibility

It is generally accepted that there is a significant level of individual variability in an athlete's concussion susceptibility (Kutcher and Eckner, 2010). In support, there is no definitive biomechanical force threshold required to induce a concussion (Guskiewicz and Mihalik, 2011). This suggests that some athletes require lower impact thresholds to sustain a concussion, while other athletes are resilient to impact forces of greater magnitudes. The individual variability associated with concussion risk warrants an investigation into the possible genetic influence on concussion susceptibility.

There are several ways to investigate the influence of candidate gene polymorphisms on concussion susceptibility. One could compare the proportion of concussed individuals, or the incidence of concussion, between candidate genotype groups (Knowles et al., 2006; Brooks and Fuller, 2006). In addition, one could also compare the prevalence of a suspected risk genotype within concussed and non-concussed groups. In addition, concussion injuries can be recorded either prospectively, by continued monitoring of athletes throughout one or more seasons, or retrospectively, through an examination of an athlete's concussion history. In sports epidemiology, prospective cohorts provide the most robust evidence, as they more accurately record exposure and reduce the risk of recall bias (Fuller et al., 2006).

In the absence of a published genome-wide association study (GWAS) for concussion susceptibility, the body of genetic exploration consists of candidate gene association studies. To date, nine candidate gene association studies have investigated the role of polymorphisms in concussion susceptibility (Table 16.1). In these studies, genes are selected based on their involvement in biological pathways relevant to a concussion.

A recent systematic review by Panenka et al. (2017) proposed three potential mechanisms by which genetic variation may influence concussion susceptibility (Panenka et al., 2017). Firstly, genetic modulation of the underlying neuroanatomical or neurophysiological properties of an individual may influence their resilience to neurotrauma (Panenka et al., 2017). The majority of the investigated candidate genes falls into this category and are involved in the structural properties or physiological response to concussion. Specifically, six studies have investigated polymorphisms in the *apolipoprotein E* (*APOE*) gene (Abrahams et al., 2018; Kristman et al., 2008; Terrell et al., 2008, 2018;

Table 16.1 A summary of the genetic polymorphisms previously investigated for an association with concussion susceptibility in sport

Study	Polymorphism(s)	Study design	Study population	Concussions (*n*)	Results
Kristman et al. (2008)	*APOE* isoforms	Prospective cohort	Canadian collegiate athletes (52% male; multiple sports; ancestries not reported; age: 20.5 ± 2.4 years)	28 (Medically diagnosed)	No association between *APOE* isoforms and prospective concussion risk
Terrell et al. (2008)	*APOE* isoforms *APOE* rs405509 T > G *Tau* rs2258689 T > C *Tau* rs10445337 T > C	Cross-sectional	American collegiate athletes (82% male American football, 9% male soccer, and 9% female soccer athletes; 51% white, 41% black, 8% other ancestries; concussed age: 19.8 ± 1.4 years, non-concussed age: 19.9 ± 1.7 years)	72 (Self-reported)	Athletes with the *APOE* rs405509 T/T genotype were three times more likely to have a concussion history compared to the G/G genotype (OR: 2.8, 95% CI: 1.1–6.9). None of the other polymorphisms showed an association with concussion history
Tierney et al. (2010)	*APOE* isoforms *APOE* rs405509 T > G *APOE* rs429358 C > T *APOE* rs7412 T > C	Cross-sectional	American collegiate athletes (83% male American football and 17% female soccer athletes; ancestries not reported; age: 19.7 ± 1.5 years)	48 (Self-reported)	There were no association between the *APOE* isoforms or *APOE* rs405509 and self-reported concussion history. Carriers of the *APOE* rs405509 T allele were at higher odds of reporting two or more concussions (OR: 8.4, 95% CI: 1.0–68.8, $P = 0.04$). Carriers of the three minor alleles (*APOE* isoforms ε2 and ε4 and the *APOE* rs405509 T allele) were 10 times more likely to have a history of concussion (OR: 9.9, 95% CI: 1.0–96.6, $P = 0.05$)
McDevitt et al. (2011)	*NEFH* rs165602 A > C	Case control	American collegiate athletes (Male American football and female soccer athletes; ancestries not reported; age: 19.5 ± 1.4 years)	48 (Self-reported)	No association between *NEFH* rs165602 and concussion history

Terrell et al. (2018)	*APOE* isoforms *APOE* rs405509 T > G *Tau* rs2258689 T > C *Tau* rs10445337 T > C *IL-6* rs1800796 G > C *IL-6R* rs2228145 A > C	Prospective cohort	American collegiate athletes (89% male; multiple sports; 59% white, 35% black, and 5.6% "other" ancestries; age: 19.7 ± 1.5 years)	133 (Medically diagnosed)	The *APOE* ε4 isoform was associated with reduced concussion risk (OR 0.6, 95% CI: 0.4–1.0; $P=0.04$). The *IL-6R* rs2228145 C/C genotype was associated with a three-fold greater concussion risk (OR 3.5, 95% CI: 1.6–7.7; $P=0.002$). There was no association between the *APOE* rs405509, *Tau* rs2258689, *Tau* rs10445337, or *IL-6* rs1800796 and concussion risk
Abrahams et al. (2018)	*APOE* isoforms *APOE* rs405509 T > G *APOE* rs429358 C > T *APOE* rs7412 T > C	Cross-sectional	South African rugby union athletes (Male; white ancestry; non-concussed age: 19.2 ± 3.5 years and concussed age: 20.5 ± 4.4 years)	160 (Self-reported)	The *APOE* rs405509 T/T genotype was under-represented in those with a concussion history (OR: 0.6, 95% CI: 0.3–1.0, $P=0.043$). The inferred *APOE* (rs405509-ε2/ε3/ε4) T-ε3 haplotype was under-represented in those with concussion ($P=0.042$). The G-ε3 *APOE* haplotype was over-represented in those with a concussion history ($P=0.021$). There was no difference in the *APOE* isoform frequencies between those with and without a concussion history
Mc Fie et al. (2018b)	*COMT* rs4680 Met/Val 5-HTTLPR & *SLC6A4* rs25531	Cross-sectional	South African rugby union athletes (Male; white ancestry; age: 20.4 ± 4.5 years)	163 (Self-reported)	The *COMT* rs4680 Val/Val was over-represented in those without a concussion history (OR: 2.0, 95% CI: 1.2–3.6, $P=0.013$). The 5-HTTLPR low expressing group was over-represented in those with a concussion history (OR: 2.0, 95% CI: 1.1–3.9, $P=0.032$)

Continued

Table 16.1 A summary of the genetic polymorphisms previously investigated for an association with concussion susceptibility in sport—cont'd

Study	Polymorphism(s)	Study design	Study population	Concussions (*n*)	Results
Cochrane et al. (2018)	*APOE* isoforms *APOE* rs405509 T > G DRD2 rs1800467 C/T *COMT* rs4680 Met/Val	Cross-sectional	American collegiate athletes (74% male; multiple sports; white, black and "other" ancestries; age: 19.0 ± 1.3 years)	60 (Self-reported)	There were no associations between genotypes and concussion history
Abrahams et al. (2019)	*Tau* rs2435211 C > T Tau rs2435200 G > A	Cross-sectional	South African rugby union athletes (Male; white ancestry; non-concussed age: 19.2 ± 3.5 years and concussed age: 20.5 ± 4.4 years)	163 (Self-reported)	The rs2435200 A/A genotype was over-represented (OR: 0.3, 95% CI: 0.1–1.0, $P = 0.033$) and the A/G genotype under-represented (OR: 2.3, 95% CI: 1.1–5.0, $P = 0.024$) in senior athletes without a concussion history, in comparison to athletes with a history of multiple concussions. The inferred *Tau* (rs2435211–rs2435200) T-G haplotype was under-represented in athletes without a concussion history, compared to those with a concussion history ($P = 0.031$)

OR, Odds ratio; *95% CI*, 95% confidence interval; *APOE*, Apolipoprotein E; *Tau*, microtubule associated protein tau; *NEFH*, Neurofilament heavy; *IL-6*, Interleukin 6; *IL-6R*, IL-6 receptor; *COMT*, Catechol-*O*-methyltransferase; *SLC6A4*, solute carrier family 6 member 4; *5-HTTLPR*, serotonin-transporter-linked polymorphic region; *DRD2*, dopamine receptor D2.

Madura et al., 2016; Tierney et al., 2010) (Table 16.1), which is implicated in neuronal structural integrity (Nathoo et al., 2003; Heise et al., 2011; Persson et al., 2006), the innate immune response (Vitek et al., 2009), and nervous tissue repair (Crawford et al., 2009). In addition, several studies have investigated the influence of polymorphisms in the *microtubule-associated protein tau* (*Tau*) gene (Abrahams et al., 2019; Terrell et al., 2008, 2018) (Table 16.1), which encodes the tau protein. Tau proteins act to assemble and stabilize neuronal microtubules (Mietelska-Porowska et al., 2014) and their dysfunction is strongly linked to pathology and degeneration of neurons (Frost and Feany, 2015; Mietelska-Porowska et al., 2014). One study examined polymorphisms in the *neurofilament heavy protein* (*NEFH*) gene (McDevitt et al., 2011), which influences neuronal cytoskeleton formation and integrity (Hisanaga and Hirokawa, 1988). The neuronal cytoskeleton is also important in resisting physical strain (Wagner et al., 2007). Another study investigated the *interleukin 6* (*IL-6*) and *interleukin 6 receptor* (*IL-6R*) genes (Terrell et al., 2018), which are key regulators of the neuroinflammatory response (Spooren et al., 2011).

Secondly, according to the aforementioned systematic review of Panenka et al. (2017), genetic variation could influence behavior that subsequently could modify the likelihood of concussion, such as impulsive, risk-taking, or aggressive behaviors (Panenka et al., 2017). Polymorphisms in the *catechol-O-methyltransferase* (*COMT*) and *solute carrier family 6 member 4* (*SLC6A4*) genes have previously been associated with risk-taking and impulsive behaviors (Balestri et al., 2014; Dalley and Roiser, 2012; Amstadter et al., 2012) and were therefore ideal candidates for investigations with concussion susceptibility (Mc Fie et al., 2018b; Cochrane et al., 2018). Polymorphisms in the *dopamine receptor D2* (*DRD2*) gene have also been linked to personality trait scores (Balestri et al., 2014; Smillie et al., 2010), as well as cognitive outcomes following brain injury (Failla et al., 2015; McAllister et al., 2008) and were therefore also examined in relation to concussion (Cochrane et al., 2018). Thirdly, genetic polymorphisms may alter the risk of comorbid conditions, which in turn might modulate concussion susceptibility, for example, attention deficit with hyperactivity disorder (Panenka et al., 2017). To date, no studies have investigated genetic variants implicated in comorbid conditions and thus this is an area that would need further investigation. The following subsections will now review the available evidence of each of these candidate genes in more detail.

16.2.1 Apolipoprotein E

The apolipoprotein E (ApoE) is a lipid transporter in the central nervous system. It has been proposed that the presence of functional *APOE* polymorphisms might alter the ability of neurons to adapt and recover from mechanical stress, and may thereby increase the risk of concussion in athletes exposed to regular head impacts (Tierney et al., 2010). The *APOE* gene has three isoforms, namely ε2, ε3, and ε4. The ε3 allele is the most common

allele across populations (60%–70%), followed by the ε4 (15%–20%), and ε2 (5%–10%) alleles (Fazio et al., 2007). The ε4 allele is associated with deficient cerebrovascular integrity following injury (Smith et al., 2006) and increased beta-amyloid affinity, potentially causing increased neural plaque aggregation (Strittmatter et al., 1993). The ε4 allele has been associated with poor outcome following TBI (Alexander et al., 2007; Zhou et al., 2008) and increased risk of several neurodegenerative disorders (Maiti et al., 2015; Alexander et al., 2007; Zhou et al., 2008).

Six studies have investigated the *APOE* isoforms and, of those, five found no significant association with sport-related concussion susceptibility (Kristman et al., 2008; Terrell et al., 2008, 2018; Tierney et al., 2010; Abrahams et al., 2018). Specifically, a prospective study including 28 concussions found no difference in concussion risk in collegiate ε4 allele carries after adjusting for sex, weight, height, and team type (Kristman et al., 2008). A retrospective cross-sectional study of male and female collegiate athletes ($n=195$) participating in American football and soccer, respectively, reported no disparity in self-reported concussion history between *APOE* isoform carriers (Terrell et al., 2008). Tierney et al. (2010) further found no association between *APOE* isoforms and concussion history in a cohort of 163 male football and 33 female soccer collegiate athletes (Tierney et al., 2010). There was also no association between *APOE* isoforms and self-reported concussion history in a cohort of 250 male and female collegiate athletes (multiple sports) (Cochrane et al., 2018). A recent retrospective study of rugby union players similarly found no difference in *APOE* isoform frequencies between those with and without a self-reported history of concussion (Abrahams et al., 2018).

Surprisingly, a large multicenter prospective study reported that the *APOE* ε4 isoform was associated with decreased concussion risk (OR 0.6; 95% CI: 0.4–1.0; $P=0.04$) (Terrell et al., 2018), contrary to the hypothesis that *APOE* ε4 would increase concussion risk based on its detrimental association with brain injury outcome (Tierney et al., 2010). The non-concussed and concussed groups differed in history of previous concussion, history of previous concussion with loss of consciousness, sex, main sport, and years playing main sport. However, including these potential confounding variables into a logistic regression did not alter the association between *APOE* ε4 and concussion risk.

The combination of the *APOE* rs429358 C > T and rs7412 T > C single nucleotide polymorphisms (SNPs) dictate the *APOE* isoform of an individual (Zannis et al., 1981). For example, the rs429358 C allele in combination with the rs7412 C allele produces the ε4 isoform. Two studies have investigated the independent association between these individual polymorphisms and concussion history and both studies found no association (Tierney et al., 2010; Abrahams et al., 2018) (Table 16.1).

Four studies have examined the functional *APOE* rs405509 T > G SNP (Table 16.1), which is located in the promoter region of the *APOE* gene. The T allele of this

polymorphism is linked with decreased *APOE* transcription and expression, in comparison to the G allele (Artiga et al., 1998). The T allele is also associated with increased risk of adverse TBI outcomes and neurodegenerative disorders (Heijmans et al., 2002; Reuter-Rice et al., 2018). American football and soccer athletes carrying the *APOE* rs405509 T/T genotype were three times more likely to report a history of concussion (OR: 2.8, 95% CI: 1.1–6.9) in a retrospective cross-sectional study (Terrell et al., 2008). Conversely, a subsequent cross-sectional study found no association between the *APOE* rs405509 genotype and self-reported concussion history (Tierney et al., 2010). However, the *APOE* rs405509 T allele carriers were at greater risk of sustaining two or more concussions (OR: 8.4, 95% CI: 1.0–68.8, $P=0.04$) and carriers of the three *APOE* rare alleles (*APOE* rs405509 T allele, isoform ε2, and isoform ε4) were 10 times more likely to report a history of concussion (OR: 9.9, 95% CI: 1.0–96.6, $P=0.05$). Although, the numbers of participants reporting two or more concussions ($n=9$) and carrying all three of the rare alleles ($n=4$) were low, which might have increased the risk of false positive findings.

In contrast, the *APOE* rs405509 T/T was overrepresented in rugby union players without a self-reported history of concussion, in comparison to those with a previous concussion (OR: 0.6, 95% CI: 0.3–1.0, $P=0.043$) (Abrahams et al., 2018). In addition, the combination of the *APOE* rs405509 T allele and the ε3 isoform, represented as an inferred haplotype, was also overrepresented ($P=0.042$), while the G-ε3 inferred haplotype was underrepresented ($P=0.019$), in those without a concussion history (Abrahams et al., 2018). A recent and large multicenter prospective study that included 1056 athletes found no association between *APOE* rs405509 and concussion risk in collegiate athletes (Terrell et al., 2018).

In summary, the majority of studies indicated that the *APOE* isoforms do not influence sport-related concussion susceptibility. However, evidence from a large prospective cohort suggested that the ε4 isoform might, in fact, reduce concussion risk. Therefore, the effect of *APOE* isoforms is at this point is still somewhat unclear. The current evidence regarding the *APOE* rs405509 SNP is also conflicting, with studies showing associations between both the G and T alleles and elevated concussion risk, or finding no significant relationship.

16.2.2 Tau

The *Tau* gene encodes tau proteins and more than 40 *Tau* polymorphisms have been associated with neurodegenerative or brain pathologies (Wolfe, 2009; Dujardin et al., 2015). There is a growing body of evidence that an increased vulnerability to a Tau-associated neurodegenerative response may increase an individual's susceptibility to sustaining multiple concussions and debilitating long-term deficits such as depression and dementia (Mez et al., 2017).

Three studies investigated the role of *Tau* polymorphisms in concussion susceptibility (Table 16.1). A large prospective cohort and a cross-sectional study of collegiate athletes both found no association between the *Tau* rs2258689 T > C and *Tau* rs10445337 T > C polymorphisms and concussion susceptibility (Terrell et al., 2008, 2018). However, these two *Tau* polymorphisms (rs2258689 and rs10445337) are considered nonpathogenic and the motivation for their selection has been questioned (Panenka et al., 2017).

Abrahams et al. (2018) investigated *Tau* rs2435211 C > T and *Tau* rs2435200 G > A for an association with concussion history in a group of South African rugby players (Abrahams et al., 2019). The selection of these *Tau* SNPs was based on their previous links to neurodegenerative conditions. Specifically, the *Tau* rs2435211 genotype was correlated with elevated tau protein levels in cerebrospinal fluid (Martiskainen et al., 2015), which is an indication of neurodegenerative pathology (Mez et al., 2017), while the rs2435200 SNP was associated with Parkinson's Disease risk (Martiskainen et al., 2015). Abrahams et al. (2018) reported that the rs2435200 A/A genotype was overrepresented (OR: 0.3, 95% CI: 0.1–1.0, $P=0.033$), and the A/G genotype underrepresented (OR: 2.3, 95% CI: 1.1–5.0, $P=0.024$), in senior (>18-years old) rugby athletes without a history of concussion ($n=66$), in comparison to senior athletes with a history of multiple concussions ($n=57$) (Abrahams et al., 2019). The authors then combined the rs2435211 and rs2435200 genotype data to create inferred *Tau* haplotypes. The T–G inferred *Tau* haplotype was found to be underrepresented in the athletes without a concussion history, compared to those with a concussion history ($P=0.031$). The results of this study implicate *Tau* pathways in the etiology of concussion and specifically in the risk of multiple concussions in senior athletes. However, a limitation of this study was that retrospective self-reported concussion histories were used as the main outcome measure and the study including small genotype sample sizes when the cohort was divided into age (senior and junior) and concussion history groups. More research is therefore required to explore these and other functionally significant *Tau* polymorphisms and their impact on concussion susceptibility and later life clinical complications.

16.2.3 Neurofilament heavy

Neurofilament heavy proteins form part of the cytoskeleton and contribute to its overall integrity (Hisanaga and Hirokawa, 1988). The *NEFH* gene encodes these neurofilament heavy proteins. One study investigated the role of the *NEFH* rs165602 A > C polymorphism in concussion but found no association between the polymorphism and concussion history (Table 16.1) (McDevitt et al., 2011). The study was, however, limited by small sample size ($n=48$ concussed athletes) and a low minor allele frequency, which may have appreciably limited statistical power. Further, adequately powered studies are required to more thoroughly investigate this polymorphism and gene.

16.2.4 Interleukin 6 and Interleukin 6 receptor

Interleukin 6 (IL-6) is a pleiotropic cytokine with a variety of functions in different tissues but has been referred to as the mental cytokine due to its fundamental proinflammatory role in the central nervous system after injury (Spooren et al., 2011). The neuroinflammatory response is implicated in concussion pathophysiology (Patterson and Holahan, 2012; Smith et al., 2013) and therefore IL-6 might also have a significant influence on concussion etiology.

One study has investigated the role of *IL-6* and *IL-6R* polymorphisms in prospective concussion risk (Terrell et al., 2018) (Table 16.1). The large prospective cohort study reported a significant association between the *IL-6R* rs2228145 SNP and concussion risk. Specifically, the *IL-6R* rs2228145 C/C genotype was associated with a three-fold greater concussion risk compared to other genotypes (OR 3.5; 95% CI: 1.6–7.7; $P=0.002$). No association was noted between the *IL-6* rs1800796 polymorphism and concussion susceptibility.

The *IL-6R* rs2228145 C allele is linked with increased *IL-6R* expression and an elevated interleukin-6 mediated inflammatory response (Ferreira et al., 2013). The authors thus postulated that the association between the *IL-6R* rs2228145 C/C genotype and heightened concussion risk might be due to an increased early inflammatory response, which might affect cognition to produce the symptoms of concussion (Terrell et al., 2018). The biological relevance of the neuroinflammatory response and the novel association presented by Terrell et al. (2018) warrants further investigation into the influence of the *IL-6R* rs2228145 polymorphism, as well as other neuroinflammatory-associated genes, in concussion.

16.2.5 Catechol-*O*-methyltransferase

Catechol-*O*-methyltransferase (COMT) is an enzyme responsible for the degradation of several neurotransmitters, particularly dopamine, from the synaptic cleft. COMT enzyme activity, therefore, has a modulating effect on dopaminergic signaling. Dopamine is a crucial central nervous system neurotransmitter, with widespread involvement in the movement, cognition, personality, and behavior (Greengard, 2001).

The *COMT* rs4680 G>A SNP causes an amino acid change from valine (Val) to methionine (Met) at codon 158 of the *COMT* gene. Val allele carriers reportedly have a three to four-fold greater COMT enzyme activity compared to the Met allele carriers (Chen et al., 2004). The *COMT* rs4680 polymorphism has been shown to have a significant effect on prefrontal cortex dopaminergic neurotransmission (Meyer-Lindenberg et al., 2006; Forbes et al., 2009) and has also been associated with a variety of cognitive, psychiatric, behavioral, and personality traits (Stein et al., 2005; Montag et al., 2012; Goldman et al., 2009).

As mentioned earlier, a possible mechanism of genetic influence on concussion susceptibility is through the modulation of personality traits or behaviors (Panenka et al., 2017). Increased concussion susceptibility has been correlated with elevated impulsive, risk-taking, and aggressive traits (Beidler, 2016; Hollis et al., 2009; Gerberich et al., 1987). The *COMT* rs4680 polymorphism has also been associated with risk-taking and impulsive behaviors (Amstadter et al., 2012; Soeiro-De-Souza et al., 2013; Malloy-Diniz et al., 2013), while *COMT* expression has been linked to aggressive behaviors (Volavka et al., 2004; Gogos et al., 1998; Kulikova et al., 2008).

The involvement of *COMT* rs4680 in the aforementioned personality traits was the motivation for a study investigating the association between *COMT* rs4680 and self-reported concussion history in a cohort of South African rugby players ($n=303$) (Mc Fie et al., 2018b). The study reported that rugby players with the *COMT* rs4680 Val/Val genotype were overrepresented in those without a concussion history (31%, $n=41$) in comparison to those with a concussion history (17%, $n=27$, OR: 2.0, 95% CI: 1.2–3.6, $P=0.013$). The Val allele was previously linked to reduced impulsive and risk-taking measures (Malloy-Diniz et al., 2013; Soeiro-De-Souza et al., 2013) and thus the authors proposed that the modulating effect of *COMT* rs4680 on concussion susceptibility might have been due to a modulation of impulsive or risk-taking behaviors. However, investigation is still required to identify whether, or how, the *COMT* rs4680 genotypes affect playing style on the rugby field, and to what extent risky or dangerous behavior contributes to concussion in rugby. The study was also limited by recording retrospective, self-reported concussion histories and thus might have been influenced by recall bias.

A subsequent retrospective cross-sectional study also investigated the association between the *COMT* rs4680 polymorphism and self-reported concussion history (Cochrane et al., 2018). The study reported no significant association between *COMT* rs4680 genotypes and self-reporting concussion history in a cohort of collegiate athletes ($n=250$, multiple sports). Both studies investigating this *COMT* polymorphism were limited by relying on self-reported concussion histories and thus the findings regarding *COMT* rs4680 need to be interpreted with caution until replicated by subsequent prospective studies.

16.2.6 Dopamine receptor D2

Dopamine elicits its postsynaptic activity through binding to one of five receptor types. Of the five receptors, the dopamine receptor D2 (DRD2) is implicated in the regulation of locomotor activity, learning and memory, reward mechanisms, personality trait scores, and outcome following brain injury (Failla et al., 2015; McAllister et al., 2008; Balestri et al., 2014; Smillie et al., 2010; Beaulieu and Gainetdinov, 2011). Polymorphisms in the *DRD2* gene are thus noteworthy candidates for concussion etiology.

One study has investigated the association between the functional *DRD2* rs1800497 C/T polymorphism and self-reported concussion history in collegiate athletes but found no significant association (Cochrane et al., 2018) (Table 16.1). However, there were only 60 athletes with a prior concussion, which may have limited statistical power. The study also primarily aimed to investigate this polymorphism in relation to cognitive performance on a concussion neurocognitive test battery, rather than concussion history, which was a secondary aim.

16.2.7 Solute carrier family 6 member 4 gene

Serotoninergic activity is implicated in a variety of personality traits, including harm avoidance, anxiousness, impulsivity, and risk-taking propensity (Balestri et al., 2014; Dalley and Roiser, 2012). The serotonin transporter is the primary mechanism by which serotonin is removed from the synaptic cleft, and thus its functional efficiency can potentially affect the duration and intensity of serotonin neurotransmission (Hariri and Holmes, 2006). The serotonin transporter is encoded by the *SLC6A4* gene. Within the gene is the serotonin-transporter-linked polymorphic region (5-HTTLPR), a 43 base pair (bp) insertion or deletion located in an upstream transcriptional control region. The 5-HTTLPR has a long allele (L), comprising of 16 repeat units, and a short allele (S) with 14 repeat units (Heils et al., 1996). Within the *5*-HTTLPR is the *SLC6A4* rs25531 A > G SNP that modulates the functional effect of 5-HTTLPR (Hu et al., 2006). The 5-HTTLPR and the *SLC6A4* rs25531 SNP are usually analyzed together as a single genotype. The 5-HTTLPR has been extensively studied and implicated in a number of personality traits, including anxiety (Sen et al., 2004), harm avoidance (Munafò et al., 2005; Sen et al., 2004), neuroticism (Munafo et al., 2009), impulsivity (Stoltenberg et al., 2012; Walderhaug et al., 2010), and aggression (Munafò et al., 2003).

A cross-sectional study investigated the influence of the combined 5-HTTLPR and *SLC6A4* rs25531 genotype on self-reported concussion history in a cohort of South African rugby players (Mc Fie et al., 2018b). The 5-HTTLPR low expressing group (S_A/S_A genotype) was overrepresented in those with a concussion history when all levels of play (high school, senior amateur, and senior professional athletes) were considered (OR: 2.0, 95% CI: 1.1–3.9, $P=0.032$). A separate analysis in each level of play established that the association was only evident in the high school cohort (OR: 3.4, 95% CI: 1.2–9.7, $P=0.021$). The high school 5-HTTLPR low and intermediate (S_A/L_A, S_A/L_G, L_A/L_G, and L_G/L_G) expressing groups also displayed decreased "harm avoidance" ($P=0.009$), "anticipatory worry" ($P=0.041$), and "fear of uncertainty" ($P<0.001$). This finding led authors to propose that the increased susceptibility in the 5-HTTLPR low expressing group might have been due to decreased harm avoidance behaviors. However, similar to the previous findings of *COMT* rs4680, the exact influence the 5-HTTLPR genotype has on the on-field behavior of rugby players and the relationship to concussion risk still

needs to be elucidated. In addition, future studies should incorporate larger sample sizes to fully investigate the potentially intricate relationships between personality traits, genetic polymorphisms, and concussion susceptibility.

16.3 Summary of the genetics of concussion susceptibility

A total of 13 polymorphisms, in six genes, have been investigated for an association with sport-related concussion susceptibility (Table 16.1). *APOE* is the most studied gene, but the evidence supporting its role in concussion susceptibility remains conflicting. Currently, there is some early evidence linking *Tau* polymorphisms to concussion risk, however, the number of investigated polymorphisms should be expanded to fully elucidate the potential role of *Tau* genetic variation in concussion. One study investigated a polymorphism implicated in neuronal cytoskeleton integrity but found no association (McDevitt et al., 2011). A large prospective study found an association between an *IL-R* polymorphism and concussion incidence, highlighting a potential role of neuroinflammatory genes in concussion risk (Terrell et al., 2018). Two personality-associated polymorphisms, *COMT* rs4680 and 5-HTTLPR, were associated with self-reported concussion history in rugby players. However, the behavioral effect of these polymorphisms and the mechanism of increased concussion susceptibility still require clarification. Overall, the limited number of studies investigating genetic risk factors for concussion is the most significant limitation in the literature. Additionally, several methodological concerns further limit the interpretation and implication of findings.

The lack of prospective cohort designs is a significant limitation in the current body of evidence. Of the nine included studies, only two prospectively recorded concussions, while the remaining seven studies asked participants to retrospectively self-report previous concussions (Table 16.1). Without clear concussion definitions and diagnostic guidelines, studies investigating self-reported concussion histories are especially vulnerable to athlete recall inaccuracies (Robbins et al., 2014). Several studies have reported that athletes have insufficient knowledge of concussion symptoms, which would influence their ability to accurately identify, report, and recall concussions (Cournoyer and Tripp, 2014; Register-Mihalik et al., 2013; Kaut et al., 2003).

A prospective cohort design is considered the gold standard to investigate injury risk (Fuller et al., 2006). Unfortunately, prospective concussion surveillance can be challenging due to the financial and human resources required. For example, the concussion incidence in youth soccer is estimated to be 0.23/1000 athlete exposures (Pfister et al., 2016). Therefore, in order to prospectively record 100 concussion events, one would have to monitor approximately 434,783 athlete exposures. Genetic association studies ideally need sample sizes in the hundreds to thousands to effectively investigate subtle genetic effects and interactions (Gauderman, 2002). In order to obtain these numbers, multicenter collaborations are required. The one genetic association study that successfully

employed a large-scale prospective cohort design included 23 university athletic programs that recruited a total of 2685 participants and recorded 133 concussions over a period of 10 years (Terrell et al., 2018).

Population stratification is the inherent variation in allele frequencies between population groups caused by ancestral differences. Population stratification has significant confounding effects on genetic association studies (Freedman et al., 2004; Thomas and Witte, 2001). Six of the nine genetic association studies assessing concussion risk included mixed population cohorts, and therefore population stratification effects might have significantly affected results. In addition, a number of intrinsic and extrinsic factors are suggested to influence concussion susceptibility (Abrahams et al., 2014). Few of the studies reported that their non-concussed and concussed groups were adequately matched for possible confounding factors, such as age, previous concussion history, and playing exposure. Therefore, it is unclear the role these confounding factors may have had on the observed genetic associations.

To date, few of the aforementioned positive findings have been consistently replicated in subsequent studies. For example, Terrell et al. (2008) reported an association between the *APOE* rs405509 polymorphism and concussion history (Terrell et al., 2008). Since that publication, three studies have investigated *APOE* rs405509, with studies reporting either no association or conflicting associations (Tierney et al., 2010; Abrahams et al., 2018; Terrell et al., 2018). However, it is unclear to what extent methodological limitations might have increased the risk of false positives in initial studies, or false negatives in replication studies. A meta-analysis of genetic association studies found that it is common for the initial study of a novel genetic marker to show a stronger association and only moderately correlate with subsequent replication studies (Ioannidis et al., 2001). Therefore, genetic associations presented in only a single study need to be interpreted with caution until subsequent replication studies have been conducted.

A concussion is a multifactorial condition and is most likely influenced by a variety of genes functioning in different domains. Previous studies have predominantly focused on genes that are proposed to modulate underlying neurophysiological and neuroanatomical properties (*APOE*, *Tau*, and *NEFH*), while there is also some emerging investigation of personality associated genetic risk factors for concussion (*COMT*, *SLC6A4*, and *DRD2*). To our knowledge, no study has probed the role of genetic polymorphisms that alter the risk of premorbid conditions that then enhance the risk of concussion. The majority of studies investigate one or two candidate genes, with few studies investigating gene-gene interactions. Further exploration into genetic interactions and novel genes involved in different biological pathways will help to advance the field (Mccrea et al., 2017). In addition, there has been no published GWAS for sport-related concussion. GWAS are the ideal mechanism to identify genetic markers, but require thousands of individual genomes and significant financial, human, and computational resources. Despite these

hurdles, it is highly likely that a GWAS on concussion etiology will be successfully conducted in the forthcoming years.

A complication to studies investigating concussion susceptibility is that concussion is difficult to clinically define due to the absence of a gold-standard confirmatory diagnostic test and the nonspecificity of resulting symptoms (Patricios and Makdissi, 2015; Coggon et al., 2005). Similarly, there is also no universal definition for concussion in the research setting, resulting in the use of a number of different concussion definitions that have evolved over time (King et al., 2014). This creates a significant barrier when attempting to directly compare studies, combine results in meta-analyses, or set up multicenter collaborations. In addition, a number of the included studies failed to disclose the definition used to identify concussions.

In conclusion, the current literature on genetic risk factors for sport-related concussion is heavily limited by the shortage of investigating studies and several methodological concerns. However, with the ever increasing interest in identifying biomarkers for concussion (Mccrea et al., 2017) and the rapid evolution of technology, it is likely that this void will soon be filled. These future studies need to utilize large-scale prospective cohort designs with medically diagnosed concussions to limit the influence of recall inaccuracies. Furthermore, a careful documentation of potential confounding factors is required. In addition, future scientific explorations should expand to include cohorts with better representation across age groups, ethnicities, sexes, and sports.

16.4 The genetics of concussion outcomes

In the majority of athletes, concussion signs and symptoms resolve within 10 days. However, for a subset comprising approximately 10%–15% of athletes, symptoms may last for weeks to months post-concussion (Mccrea et al., 2014; Cancelliere et al., 2014). Full recovery and safe return to play is only considered when all symptoms and deficits have resolved (McCrory et al., 2017). In research, the intensity or duration of symptoms and deficits are recorded as the primary concussion outcome measures. In the absence of a definitive gold-standard indicator, a variety of clinical tools are utilized to probe the severity and resolution of concussion deficits in different domains.

Identifying which athletes are at risk of prolonged post-concussion dysfunction is required to improve management strategies, avoid a premature return to play, and thus decrease the risk of severe secondary complications. There is a significant level of heterogeneity in concussion outcomes. Impact biomechanics do not predict clinical outcome (Guskiewicz and Mihalik, 2011) and, although a number of risk factors have been proposed, there are currently no accurate prognostic markers of extended deficits (Cancelliere et al., 2014). The individual variability associated with post-concussion outcomes implicates genetic variation in the emergence and resolution of concussion signs and symptoms.

The pathophysiological processes of brain injuries are divided into two classifications, namely the primary and the secondary insults. The primary insult refers to the mechanical forces transmitted to the brain on impact, while the secondary insult encompasses the subsequent biological responses. The clinical outcome depends on a combination of the primary and secondary insult severities. Therefore, genes involved in either the structural integrity and resistance to mechanical force (primary insult) or the subsequent molecular signaling pathways (secondary insult) are ideal candidates for an association with concussion outcomes. To date, eight genetic association studies have investigated concussion outcome measures (Table 16.2). Examined genes include *APOE*, *solute carrier family 17 member 7* (*SLC17A17*), *glutamate ionotropic receptor NMDA type subunit 2A* (GRIN2A), *interleukin 1β* (*IL-1B*), *IL-6*, and *caspase 8* (*CASP8*). *APOE*, as previously discussed, is involved in the innate immune response (Vitek et al., 2009), nervous tissue repair (Crawford et al., 2009), and several neurodegenerative conditions (Maiti et al., 2015; Alexander et al., 2007). *APOE* is, therefore, a prime candidate for an association with concussion outcomes. The *SLC17A7* and *GRIN2A* genes are both involved in glutamate signaling and may thus be involved in the regulation of the glutamatergic excitotoxicity observed following a concussive injury (Giza and Hovda, 2001). *IL-6* and *IL-1B* are regulators of the neuroinflammatory response (Bélanger et al., 2011), while *CASP8* is known to regulate apoptosis following brain injury (Zhang et al., 2003). Neuroinflammation and apoptosis both form part of the physiological response to concussion (Blaylock and Maroon, 2011; Lai and Todd, 2008; Conti et al., 1998; Rink et al., 1995). Each of these candidate genes will now be discussed in more detail.

16.4.1 Apolipoprotein E

APOE is quickly upregulated in neurons and glia following brain injury (Horsburgh et al., 1999) and contributes to the subsequent innate immune response, neuronal repair, and synaptogenesis (Vitek et al., 2009; Crawford et al., 2009; Fazio et al., 2007). Due to the aforementioned roles, *APOE* has been the most frequently investigated gene in TBI research. The ε4 allele has consistently been associated with poor long-term TBI outcomes (Kassam et al., 2016; Zhou et al., 2008; Lawrence et al., 2015) and neurodegenerative disorder development (Maiti et al., 2015; Alexander et al., 2007). In vitro studies have implicated the ε4 isoform in reduced neurite growth, synaptic plasticity, and protection against oxidative stress (Horsburgh et al., 2000; Kim et al., 2014).

Considering the existing TBI literature, *APOE* is an ideal candidate gene for explorations into concussion outcomes. In total, four studies have investigated *APOE* polymorphisms and concussion outcomes (Table 16.2). Three of those studies examined the *APOE* isoforms, while one study included the *APOE* rs405509 G > T, rs429358 T > C, and rs7412 C > T polymorphisms.

Table 16.2 A summary of the genetic polymorphisms previously examined for an association with sport-related concussion outcomes

Study	Polymorphism	Study design	Study population	Sample size (*n*)	Results
McDevitt et al. (2011)	*NEFH* rs165602 A > C	Case control	American collegiate athletes (Male American football and female soccer athletes; ancestries not reported; age: 19.5 ± 1.4 years)	48 (Self-reported)	No association was evident between *NEFH* rs165602 and concussion symptom or return to play durations
McDevitt et al. (2015)	*GRIN2A* rs3219790 VNTR	Case series	American athletes (74% male; sport type not reported; 52% Caucasian, 14% African American, 3% Hispanic, 1% Native American, 2% mix, and 25% ancestry not reported; age: 19.5 ± 6.0 years)	87 (Medically diagnosed)	Athletes with at least one (OR: 2.1; Wald $\chi^2 = 4.209$, $P = 0.048$) or two (OR: 6.0; Wald $\chi^2 = 4.082$, $P = 0.043$) *GRIN2A* rs3219790 long alleles were more likely to take longer than 60 days to return to play
Merritt and Arnett (2016)	*APOE* isoforms	Case series	American collegiate athletes (83% male; multiple sports; 71% Caucasian and 29% other ancestries; age ε4 carriers: 19.9 ± 1.4 years and age ε4 noncarriers: 20.0 ± 1.6 years)	42 (Medically diagnosed)	*APOE* ε4 carriers reported higher symptoms scores (Cohen's $d = 0.73$, $P < 0.05$) and were more likely to report physical ($\chi^2 = 5.95$, $P = 0.015$; Nagelkerke's $R^2 = 0.2$, OR: 5.3, 95% CI: 1.3 to 20.8) and cognitive ($\chi^2 = 5.45$, $P = 0.020$; Nagelkerke's $R^2 = 0.2$, OR: 4.8, 95% CI: 1.2–18.4) symptoms than noncarriers
Merritt et al. (2016)	*APOE* isoforms	Case series	American collegiate athletes (83% male; multiple sports; 71% Caucasian and 29% other ancestries; age ε4 carriers: 20.0 ± 1.4 years and age ε4 noncarriers: 20.0 ± 1.5 years)	45 (Medically diagnosed)	A greater proportion of the *APOE* ε4 allele carriers reported headaches (75%, $n = 12$) compared to than noncarriers (41%, $n = 12$, $\chi^2 = 4.68$, $P = 0.030$). The ε4 carriers reported a greater headache symptom scores (median: 1.5) than noncarriers (median: 0, $U = 141.5$, $P = 0.23$, $r = 0.34$)
Madura et al. (2016)	*SLC17A7* rs74174284 C > G	Case series	Athletes (Mixed sexes, multiple sports, ancestries, and ages)	40 (Medically diagnosed)	The *SLC17A7* rs74174284 C/C genotype (33.4 ± 10.2) had worse motor speed at the initial post-concussion assessment compared to other genotypes (41.6 ± 7.4, $P = 0.01$), while carriers of the G allele were more likely to experience prolonged recovery times ($P = 0.0179$)

Merritt et al. (2018)	*APOE* isoforms	Case series	American collegiate athletes (77% male; multiple sports; 72% Caucasian and 28% other ancestries; age ε4 carriers: 20.3 ± 1.4 years and age ε4 noncarriers: 20.3 ± 1.5 years)	60 (Medically diagnosed)	There was no association between the presence of the *APOE* ε4 isoform and post-concussion neurocognitive scores. *APOE* ε4 carriers were more likely to report three or more impaired test scores (40%, $n=8$), in comparison to the ε4 noncarriers (16%, $n=6$, $\chi^2=4.0$, $P=0.046$). Carriers of the ε4 isoform displayed greater intraindividual variability in neurocognitive test scores following concussion ($\chi^2=3.8$, $P=0.050$)
Mc Fie et al. (2018a)	*IL-1B* rs16944 C > T *IL-6* rs1800795 G > C *CASP8* rs3834129 Ins > Del	Cross-sectional	South African rugby union athletes (Male; White ancestry; age: 20.4 ± 4.5 years)	163 (Self-reported)	The proportion of participants with high symptom counts increased across the *IL-1B* rs16944 ($P=0.047$) and the *IL-6* rs1800795 ($P=0.027$) genotypes. The C–C inferred interleukin allele construct was underrepresented in those reporting a high symptom count ($P=0.002$) and prolonged symptom duration ($P=0.015$)
Abrahams et al. (2018)	*APOE* rs405509 G > T *APOE* rs429358 T > C *APOE* rs7412 C > T	Cross-sectional	South African rugby union athletes (Male; White ancestry; non-concussed age: 19.2 ± 3.5 years and concussed age: 20.5 ± 4.4 years)	160 (Self-reported)	The *APOE* rs405509 T allele was over-represented in the group that reported symptom durations greater than 1 week, (OR: 0.6, 95% CI: 0.3–1.0, $P=0.048$)

95% CI, 95% confidence interval; APOE, apolipoprotein E; *Tau*, microtubule associated protein tau; *NEFH*, neurofilament heavy; *OR*, odds ratio; *GRIN2A*, glutamate ionotropic receptor NMDA type subunit 2A; *SLC17A7*, solute carrier family 17 member 7; *VNTR*, variable nucleotide tandem repeat; *Ins*, insertion; *Del*, deletion.

Collegiate athletes carrying the *APOE* ε4 isoform ($n=15$) reported greater total self-reported symptom scores on the post-concussion symptom scale (PCSS) in comparison to noncarriers ($n=27$) (Cohen's d$=0.73$, $P<0.05$) (Merritt and Arnett, 2016). Further investigation revealed that ε4 carriers were more likely to report physical ($\chi^2=5.95$, $P=0.015$; Nagelkerke's $R^2=0.2$, OR: 5.3, 95% CI: 1.3–20.8) and cognitive ($\chi^2=5.45$, $P=0.020$; Nagelkerke's $R^2=0.2$, OR: 4.8, 95% CI: 1.2–18.4) symptoms than noncarriers. Affective and sleep symptoms were similar between the isoform groups. Logistic regression analysis calculated that the ε4 isoform predicted 18% of cognitive and 16% physical symptom variance. The study was however limited by small sample size ($n=42$, 83% male), the genetic analysis of mixed population groups ("Caucasian": 71%; "other": 29%), and self-reported symptom data. The timing of test sessions also varied greatly between participants (range: 0–72 days post-concussion).

A subsequent study by the same research group investigated whether the *APOE* isoforms were specifically associated with the presence or severity of post-concussion headaches (Merritt et al., 2016). Merritt et al. (2016) reported that the *APOE* ε4 isoform carriers were more likely to report post-concussion headaches (75%, $n=12$) than noncarriers (41%, $n=12$, $\chi^2=4.68$, $P=0.030$). Additionally, ε4 carriers reported greater headache severity scores (median: 1.5) than noncarriers (median: 0, $U=141.5$, $P=0.23$, $r=0.34$). The study additionally included a control group of non-concussed athletes ($n=43$) to investigate potential differences in baseline headache reporting. The *APOE* isoform had no influence on headache presentation in the control group, suggesting that in the absence of brain injury *APOE* isoforms have no effect on headache development. The limitations of the study are the same as the previously mentioned Merritt et al. (2016) study, as the same methodology was used.

In contrast, no differences in symptom scores, measured using the Post-Concussive Symptom Interview, were observed between ε4 allele carriers and noncarriers in a cohort of pediatric patients ($n=99$) (Moran et al., 2009). However, it must be noted that this study did not exclusively investigate sport-related concussions and additionally included mild TBI's and concussions sustained during falls (20%), transport accidents (12%), and other events (7%). Although the remaining majority of the concussions occurred during sport-participation or recreation activities (61%), the heterogeneity of injury mechanism might limit the study's comparability to those primarily investigating sport-related concussions.

One study has investigated the influence of the *APOE* isoforms on the post-concussion neurocognitive function of athletes (Table 16.2). The study by Merritt et al. (2018) incorporated both traditional paper and pencil neuropsychological tests and the Immediate Post-Concussion Assessment Testing (ImPACT) computerized battery (Merritt et al., 2018). There was no difference between ε4 carriers ($n=20$) and noncarriers ($n=37$) in post-concussion mean standardized neurocognitive scores (Merritt et al., 2018). However, when using a cut-off value of three impaired test scores (scores greater than 1.5 standard deviations below the mean) to designate participants into

"impaired" ($n=14$) and "not impaired" ($n=43$) groups, it was stated that ε4 carriers were more likely to fall into the "impaired" group (40%, $n=8$) in comparison to the ε4 noncarriers (16%, $n=6$, $\chi^2=4.0$, and $p=0.046$). In addition, the authors split participants into "high" (47%, $n=27$) and "low" (53%, $n=30$) variability groups based on the within-person variability of test scores. A greater proportion of the ε4 carriers were included in the "high" variability group (65%, $n=13$) in comparison to noncarriers (38%, $n=14$, $\chi^2=3.8$, and $P=0.050$). The authors postulated that the increased neurocognitive score variability might be a marker of less efficient cognitive processing in *APOE* ε4 carriers. Overall, the study presented partial support of an association between *APOE* isoforms and post-concussion neurocognitive function. But again, the study was limited by small sample size ($n=57$ and 77% male), analysis of mixed population groups ("Caucasian": 72%; "other": 28%), and the variability of post-concussion testing timing (range: 0–150 days). The study also appeared to require a fair amount of statistical gymnastics to produce significant associations.

The influence of *APOE* genetic variation on baseline cognition is a complication to studies investigating post-concussion neurocognitive scores. A recent study by Cochrane et al. (2018) reported that baseline neurocognitive scores differed between *APOE* isoforms on ImPACT testing (F:2.01, Wilks' $\lambda=0.91$, $P=0.03$). Studies investigating neurocognitive indices, therefore, need to incorporate individual baseline testing to control for these confounding effects.

One study has investigated other *APOE* polymorphisms and concussion outcomes. Abrahams et al. (2018) investigated the relationship between the *APOE* rs405509 G > T, rs429358 T > C, and rs7412 C > T SNPs and self-reported symptom duration in a cohort of male rugby players (Abrahams et al., 2018). In the study, participants ($n=160$; aged: 19.2 ± 3.5 years old) were split into symptom duration groups around the median duration of symptoms (one week). The *APOE* rs405509 T allele was overrepresented in the group that reported symptom durations greater than one week (51%, $n=70$), as opposed to those reporting shorter symptom durations (36%, $n=29$, OR: 0.6, 95% CI: 0.3–1.0, $P=0.048$). There were no differences in the *APOE* rs429358 and rs7412 frequencies between symptom duration groups. The main limitation of the study was that symptom duration data were retrospectively self-reported by participants. The included concussion events occurred on average 2.5 ± 2.7 years before study recruitment.

Currently, there is possibly some emerging support for the role of *APOE* polymorphisms in concussion outcomes. However, the evidence is severely restricted by the limited number of studies and methodological shortcomings of existing studies.

16.4.2 Neurofilament heavy

As mentioned previously, the *NEFH* gene is implicated in neuronal cytoskeleton integrity and was thus proposed to influence the resilience to concussion forces (McDevitt

et al., 2011; Hisanaga and Hirokawa, 1988). In addition to investigating the association between the *NEFH* rs165602 A>C polymorphism and concussion history, McDevitt et al. (2011) further analyzed the relationship between genotype and self-reported duration of concussion symptoms and the time taken to return to play (McDevitt et al., 2011). The study reported no significant associations between *NEFH* rs165602 and the duration of symptoms or return to play times. The study was, however, limited by several factors. Specifically, the cohort consisted of 48 athletes and relied on self-reported concussion outcomes. It was also not clear what signs and symptoms athletes were asked to recall or whether a symptom checklist was provided to capture this information.

16.4.3 Glutamate ionotropic receptor NMDA type subunit 2A

Following a concussion injury, the mechanical forces inflict a stretching of neuronal membranes causing an uncontrolled flux of ions through previously tightly regulated channels (Katayma et al., 1990; Giza and Difiori, 2011). Uncontrolled amounts of glutamate are released into the synapse and bind to NMDA receptors, resulting in Ca^{2+} ion influx (Giza and Hovda, 2001). Elevated intracellular Ca^{2+} levels trigger the neurons to experience excitotoxicity, which may cause damage to several cell components and lead to cell death (Momeni, 2011; Vagnozzi et al., 2007; Arundine and Tymianski, 2004).

The *GRIN2A* gene encodes for the 2A subunit of the NMDA receptor. Within the promoter region of the *GRIN2A* gene is a variable (GT)n repeat (rs3219790) that has been shown to modulate *GRIN2A* expression (Itokawa et al., 2003). Specifically, the longer alleles ($\geq$25 repeats) were associated with decreased transcription of *GRIN2A*. McDevitt et al. (2015) investigated the role of this polymorphism in concussion outcome in a cohort of athletes with diagnosed concussions ($n=87$). The study prospectively followed the time taken for each athlete to be cleared to return to play by a physician. The athletes took on average 56 days to return to play and therefore the authors designated 60 days as the cut-off between a "normal" ($n=67$) and "prolonged" recovery ($n=20$). Carriers of the *GRIN2A* rs3219790 long allele were twice as likely (OR: 2.1; Wald $\chi^2=4.209$, $P=0.048$), and the long/long homozygotes six times as likely (OR: 6.0; Wald $\chi^2=4.082$, $P=0.043$), to return to play more than 60 days post-concussion (Table 16.2) (McDevitt et al., 2015). However, the significant association was lost when the cut-off between recovery groups was adjusted to either 40 days ($P=0.109$) or 50 days ($P=0.094$).

The study did have several methodological constraints. The recovery groups were not adequately matched in sex or ancestry distributions. Females are suggested to be at greater risk of prolonged symptom durations (Miller et al., 2016). In this study, females represented 40% of the prolonged recovery group ($n=8$) and 22% of the normal recovery group ($n=15$). Ancestral differences can also have significant confounding effects in genetic association studies (Tang et al., 2005). The study was further limited by small

sample size ($n=87$) and thus findings need to be interpreted with caution. Due to the pathophysiological significance of the NMDA receptor and the preliminary evidence presented by McDevitt et al. (2015), further investigation is warranted into the role of *GRIN2A* polymorphisms in concussion outcomes.

16.4.4 Solute carrier family 17 member 7

The *SLC17A7* gene encodes for a sodium-dependent, vesicle-bound phosphate transporter involved in the transport of glutamate in neuron-rich areas. The *SLC17A7* rs74174284 C > G SNP, located in the promoter region of the *SLC17A7* gene, was associated with post-concussion symptom duration and neurocognitive function in a sample of 40 athletes (Table 16.2) (Madura et al., 2016). Carriers of the rs74174284 G allele were reportedly six times more likely to experience prolonged symptom durations ($P=0.0179$). In addition, the *SLC17A7* rs74174284 C/C genotype carriers (33.4 ± 10.2) had worse motor speed on the first post-concussion ImPACT assessment in comparison to other genotype carriers (41.6 ± 7.4, $P=0.01$). These findings provide some early support for the role of genetic variation in glutamate regulating pathways in concussion severity. However, without additional investigation, the supporting evidence remains limited.

16.4.5 Interleukin 1β and Interleukin 6

The neuroinflammatory response is initiated following concussion (Patterson and Holahan, 2012; Smith et al., 2013) and is suggested to influence concussion symptom presentation and duration (Blaylock and Maroon, 2011; Lai and Todd, 2008). The post-concussion syndrome has even be referred to as a "post-inflammatory brain syndrome" due to the significant role of inflammation in the concussion pathophysiology (Rathbone et al., 2015).

On recent study investigated the role of functional polymorphisms in the *IL-1B* and *IL-6* genes (Mc Fie et al., 2018a). IL-1B and IL-6 are the two interleukins primarily expressed following brain injury (Hopkins and Rothwell, 1995; Harrison et al., 2014) and play an important role in mediating the neuroinflammatory response (Bélanger et al., 2011). IL-1B upregulates the expression of IL-6 (Norris et al., 1994; Woodroofe et al., 1991) and both interleukins act to stimulate the neuroinflammatory response (Basu et al., 2004; Erta et al., 2012). Altered IL-1B and IL-6 expression and protein levels have been associated with neuroinflammatory variation (Almolda et al., 2014; Rothwell, 2003) and clinical outcomes following brain injury (Singhal et al., 2002; Winter et al., 2004; Clausen et al., 2011).

In the study, rugby players with a previous concussion ($n=163$) were split into self-reported concussion symptom severity groups around (i) the number of reported symptoms and (ii) the duration of symptoms (Mc Fie et al., 2018a). The proportion of

participants with high symptom counts (>5 symptoms) increased across both the *IL-1B* rs16944 (C/C: 35%; C/T: 51%; T/T: 56%; $P=0.047$) and the *IL-6* rs1800795 (C/C: 31%; C/G: 44%; G/G: 58%; $P=0.027$) genotypes (Table 16.2). There were no significant associations between genotypes and symptom duration group frequencies. Authors then additionally investigated the combined influence of the *IL-1B* and *IL-6* genotypes by creating inferred allele constructs. Unsurprisingly, the C–C inferred interleukin allele construct was underrepresented in those reporting a high symptom count (18%), in comparison to those reporting a low symptom count (36%, $P=0.002$). In addition, the C–C inferred interleukin allele construct frequency was also lower in those reporting prolonged symptom duration (>1 week, 16%), as opposed to short symptom duration (≤1 week, 34%, $P=0.015$).

These findings suggested that the *IL-1B* rs16944 and *IL-6* rs1800795 C alleles were linked to reduced concussion symptom severity. Furthermore, the inferred interleukin allele construct associations indicated that the *IL-1B* and *IL-6* polymorphisms might have had an additive modulating effect. Previous functional evidence has associated both the *IL-1B* rs16944 and *IL-6* rs1800795 C alleles with decreased protein production and signaling, which would presumably result in decreased neuroinflammatory initiation in comparison to the *IL-1B* rs16944 T and *IL-6* rs1800795 G alleles, respectively (Santtila et al., 1998; Miñambres et al., 2003). Although neuroinflammation is necessary to promote healing in the central nervous system, a prolonged or over-active response can have detrimental effects on the health of nervous tissue (Lenzlinger et al., 2001; Morganti-Kossmann et al., 2002). Therefore, the authors proposed that the reported beneficial effect of the *IL-1B* rs16944 and *IL-6* rs1800795 C alleles on concussion symptom severity might have been due to limited neuroinflammatory response.

The study did, however, have some methodological limitations that need to be taken into account when interpreting these findings. Participants retrospectively self-reported their symptom presentations from concussion events that occurred, on average, 2.5 years (std dev. 2.7 years) before study participation. Recall inaccuracies might have thus influenced the reliability of the study's outcome measures. Subsequent studies are required to further interrogate these findings.

16.4.6 Caspase 8

Neuronal apoptosis is thought to be induced and influences the pathology of concussion (Conti et al., 1998; Rink et al., 1995). Specifically, elevated levels of apoptosis are thought to increase the degree of neuronal network disruption following brain injury, resulting in more severe symptoms (Kraus et al., 2007). Caspases are a family of proteases involved in regulating apoptotic pathways (Grütter, 2000; McIlwain et al., 2013). Caspase 8 is an initiator caspase that is specifically implicated in stimulating apoptotic pathways subsequent to brain injury (Zhang et al., 2003).

The *CASP8* gene, located on chromosome two, encodes the caspase 8 protein. An insertion/deletion polymorphism (*CASP8* rs3834129 Ins > Del) in the promoter region of *CASP8* was shown to alter *CASP8* transcription (Sun et al., 2007) and thus might modify caspase 8 initiated apoptosis (Sun et al., 2007; Ji et al., 2009). Due to the suggested regulatory role in apoptotic pathways, the *CASP8* rs3834129 was investigated in a study examining symptom severity in rugby players (Mc Fie et al., 2018a). However, no associations were noted between the functional *CASP8* rs3834129 polymorphism and self-reported symptom number or duration (Table 16.2). Despite this, apoptotic pathways still warrant further investigation as modulators of concussion severity, due to their role in concussion pathophysiology.

16.5 Summary of the genetics of concussion outcome

There has been a significant scientific investigation into the genetic influence on recovery following TBI, but explorations into sport-related concussion is a relatively new field, with the first of the existing eight studies published in 2011. Similar to concussion susceptibility, *APOE* is the most scrutinized candidate gene. Three studies, from the same research group, examined *APOE* isoforms, producing some early evidence that the ε4 isoform might be associated with increased concussion symptom severity and neurocognitive difficulties (Merritt and Arnett, 2016; Merritt et al., 2016, 2018). One study investigated the *APOE* rs405509 G > T SNP and found an association between the T allele and prolonged self-reported symptom durations in a cohort of male rugby players (Abrahams et al., 2018). McDevitt et al. (2015) and Madura et al. (2016) both investigated genes implicated in glutamate signaling, namely *GRIN2A* and *SLC17A7*, respectively (McDevitt et al., 2015; Madura et al., 2016). The *GRIN2A* rs3219790 long allele and the *SLC17A7* rs74174284 G allele were both linked to prolonged return to play, suggesting that genetic modulation of glutamate might influence concussion symptom durations. Two polymorphisms in interleukin genes, namely *IL-1B* rs16944 and *IL-6* rs1800795, were independently, and in combination, associated with self-reported concussion symptom severity, producing some early evidence for the role of neuroinflammatory genes in concussion outcomes (Mc Fie et al., 2018a). From the available studies, there is some preliminary support for the hypothesis that genetic variation can influence concussion outcomes; however, the small body of evidence is further restricted by several significant methodological limitations.

The majority of available studies were limited by small sample size. Only two of the eight studies included more than 100 concussed participants (Table 16.2) and those two studies had to rely on retrospective self-reported concussion histories. Without adequately powered statistical analyses the reported positive findings need to be interpreted with great caution until replicated by larger cohorts. Ideally, studies should include prospective, medically documented concussion outcome measures. Studies that

incorporated self-reported symptoms might have been significantly affected by recall bias. Athletes' concussion symptom recognition can be less than adequate (Gourley et al., 2010), leading to further inaccuracies in symptom reports. Similar to the studies investigating concussion susceptibility, the majority of studies (six out of the eight studies) included several ancestral groups in the analyses, which might have lead to confounding population stratification effects.

There was a large variation in concussion outcome classifications and the clinical tools used to measure concussion severity (Zemek et al., 2013). Several different symptoms checklists and neurocognitive test batteries were used in the included studies. An additional complication is that few symptom checklists have been scientifically scrutinized or validated (Alla et al., 2009), while the reliability of neurocognitive test programs has also been questioned (Barlow et al., 2011; Kirkwood et al., 2009; Schatz, 2010). Historically, cognitive symptoms were disproportionately assessed and measured following a concussion. In recent years, investigations have shown the wide range of possible concussion symptoms, but symptom checklists have yet to incorporate the full spectrum of signs and symptoms. Therefore, a genetic variation that may influence cognitive symptoms preferentially may show a stronger association with symptom scores in checklists with an overrepresentation of cognitive symptoms.

There is currently no standardized definition of what constitutes a prolonged recovery time. Two studies used symptom durations of one week to differentiate normal and prolonged deficits, while another study used 60 days as their cut-off. This makes it difficult to directly compare findings between studies. Future studies assessing concussion recovery should aim to use standard injury criteria, assessment time points, and outcome measures.

To date, the genetic association studies have focused on the acute outcomes following a concussion, with an absence of investigation into the suspected permanent degenerative neurological changes. This will be an important area of future research as the concern surrounding the long-term effects of multiple concussions or sub-concussive impacts grows.

In conclusion, the field of genetic variation in concussion outcomes is still relatively in its infancy and thus definitive genetic markers are at this point unclear. The current knowledge base would be greatly expanded by the inception of multicenter collaborations utilizing prospective medical injury surveillance with standardized clinical tools and both acute and chronic outcome measures. Until that time, although promising, the existing evidence remains preliminary.

16.6 Conclusion

In recent years, the sport-related concussion has received exponentially increasing attention from both the public and scientific communities. The relative frequency and the potentially detrimental outcomes have caused a concussion to be considered a global

health concern. The heterogeneity of concussion risk and outcome has prompted novel exploration into the underlying genetic modulation of concussion etiology and, to date, 17 studies have investigated potential associations between genetic polymorphisms and concussion susceptibility or outcome. Several of these studies reported interesting and novel relationships between genetic factors and concussion. However, a number of methodological limitations and the general absence of supporting replication studies stunt the emergence of strong evidence from the available literature at this point in time. These preliminary studies do still provide a good base to guide subsequent large-scale, methodologically sound, and prospective studies that will then be able to interrogate both the previous candidate genes as well as identify novel markers.

Genetic testing has been suggested as a future screening tool in the context of sport-related concussions (Jordan, 1998). Almost three-quarters of collegiate athletes expressed interest in genetic testing to detect those at risk of poor outcomes following concussion (Hercher et al., 2016). It must be noted that genetic predisposition does not equal predetermination, but rather highlights potentially at-risk individuals. It is also likely that concussion susceptibility and outcome are polygenic and not limited to a single causative gene or polymorphism. Before a polymorphism can be considered for use in genetic screening, the association needs to be replicated in several populations with large sample sizes (Chanock et al., 2007). Therefore, the genetic associations highlighted in this chapter require further investigation, preferably in a large-scale prospective design, before the polymorphisms can be confidently linked to concussion and incorporated into a genetic screen. Hence, there is currently little support for the use of genetic testing for sport-related concussion. Despite this, the investigation of genetic polymorphisms in relation to concussion provides valuable information on the underlying mechanisms that may lead to a concussion. Furthermore, it is highly likely that, in the future, genetic profiles will inform individualized treatment and management strategies that will in turn significantly enhance the well-being of athletes (Mccrea et al., 2017).

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CHAPTER SEVENTEEN

The genetic association with exercise-induced muscle damage and muscle injury risk

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17.1 Introduction

Sporting success is the result of the relative contributions of nature (genetic) and nurture (environmental) factors. Diverse environments of sports are likely to act as agents of natural selection (Kujala et al., 2000). The relative importance of heritable (genetic) factors in shaping champions is a constant area of debate (Tucker and Collins, 2012). The current understanding suggests above 50% of the variance for an elite athlete's status can be accounted for by genetics (De Moor et al., 2007; Georgiades et al., 2017; Thomis et al., 1998). However, an athlete's potential to succeed at the highest level of sport may be determined in part by their physical resilience, such as the capability to recover, and their susceptibility to injury, particularly in regard to muscle tissue (Durand-Bush and Salmela, 2002; Reilly and Williams, 2003). For instance, highly dynamic skills require eccentric (muscle lengthening) muscle contractions in order to control the deceleration of body segments, such as the deceleration of the shaft in the late swing phase of sprinting during high-speed running in soccer (Ekstrand et al., 2011b; Opar et al., 2012; Brooks et al., 2006). This can result in disruption of the sarcomeres and the extracellular matrix (ECM) surrounding the entire skeletal muscle, muscle fascicles, and muscle fibers with three different layers, followed by delayed onset muscle soreness and tenderness, loss of strength, and an increase in muscle-specific proteins (e.g., creatine kinase and myoglobin) in the circulation.

Following this type/intensity of exercise, some individuals will experience stiff or sore muscles, which typically occurs 24–72 h after exercise, while others do not feel any muscle soreness despite performing the same relative intensity of exercise. Several factors have been reported, which contribute to these individual differences including age (Fielding et al., 1991), ethnic origin (Sherwood et al., 1996), sex (Sewright et al., 2008), training level (Balnave and Thompson, 1993), and nutrition (Howatson and van Someren, 2008).

Sports, Exercise, and Nutritional Genomics
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Nonetheless, when all these factors are taken into account, they still cannot entirely explain the considerable variation in the response to exercise-induced muscle damage (EIMD) and an emerging field of investigations suggest that genetic variation may play a critical role in influencing a person's susceptibility to EIMD (Baumert et al., 2016a). Variation in the genes encoding proteins involved in the muscle tendon unit (MTU) may alter the stiffness as well as the integrity of the MTU, and therefore are likely to affect the muscle damage response (Baumert et al., 2016b). Exorbitant strenuous exercise without adequate time to recover might promote the maladaptation to exertion known as rhabdomyolysis (Rawson et al., 2017) and the risk of muscle injury (Larruskain et al., 2018).

Detecting genetic variations linked to EIMD could help to improve the understanding of the mechanisms underpinning EIMD. To determine whether the identified SNPs related to EIMD, exertional rhabdomyolysis, and/or skeletal muscle strain injuries are expressed specifically in skeletal muscle or elsewhere, we aligned the SNPs with figures of baseline transcription levels from the genotype-tissue expression (GTEx; RRID: SCR_001618; (Consortium, 2015)) database with the current literature. This database consolidates information between genetic variation and gene expression in multiple human tissues. Of 23 identified SNPs that are associated with EIMD and skeletal muscle injury, only four (*ACTN3, CKM, TRIM63*, and *SOD2*) related genes are primarily expressed in skeletal (and cardiac) muscle tissue. Other genes, in which EIMD and muscle injury-related SNPs are located, are expressed in varying tissues with a high (*IGF2* and *HIF1A*), low (*ACE*, *CCL2*, *COL5A1*, *HGF*, *IL6 NOS3*, *MMP3 MYLK*, *SOX15*, *SPP1*, and *TNC*), or absent baseline expression (*COL2A1*, *LC30A8*, *IL1*, and *TNF*) in skeletal muscle tissue (Consortium, 2015), highlighting the complex response of varying tissues involved in EIMD and muscle injury.

In the first part of this chapter, common SNPs related to EIMD are highlighted. In the second part of this chapter, common SNPs that are associated with the maladaptation to EIMD, in particular to exertional rhabdomyolysis and muscle strain injuries, are reported.

17.2 Part I: The mechanisms underlying EIMD

Strenuous exercise can result in ultrastructural muscle damage that is manifested in Z-line disturbance as well as disruption of the muscle ECM, that is essential for muscle remodeling as well as for (lateral) force transmission (Tidball, 1991; Garg and Boppart, 2016). The disruption of the sarcomeres and the muscle ECM precipitates leucocytes around the injury site (Tidball, 2005). Within the first 24 h, neutrophils infiltrate the damaged muscle fiber followed by M1 (pro-inflammatory) and M2 (antiinflammatory) macrophages. Neutrophils and M1 macrophages play a role in removing cell debris (Tidball, 2017). Their infiltration to the injury site can lead to an activation of different degradation pathways, for example, to ubiquitin-mediated protein degradation by

attaching ubiquitin polymers to cellular debris (E1–E3 ligases) and the subsequent degradation by the 26S-proteasome complex (Fig. 17.1).

The shift from M1 to M2 macrophages is accompanied with muscle stem cell activation in both the skeletal muscle (satellite cells) and the surrounding muscle ECM (fibroblasts) (Evano and Tajbakhsh, 2018). Both activated satellite cells and fibroblasts proliferate and then migrate to the injury site along the basal lamina and the ECM. The interaction between activated satellite cells, fibroblasts and other components of the ECM is highly complex, acting cooperatively in a fine-tuned coordinated fashion to support healthy remodeling (Hughes and Blau, 1990; Yin et al., 2013; Mann et al., 2011; Murphy et al., 2011). However, extraordinary muscle damage together with insufficient recovery can lead to incomplete healing or even to long-lasting unfavorable fibrosis (Butterfield, 2010).

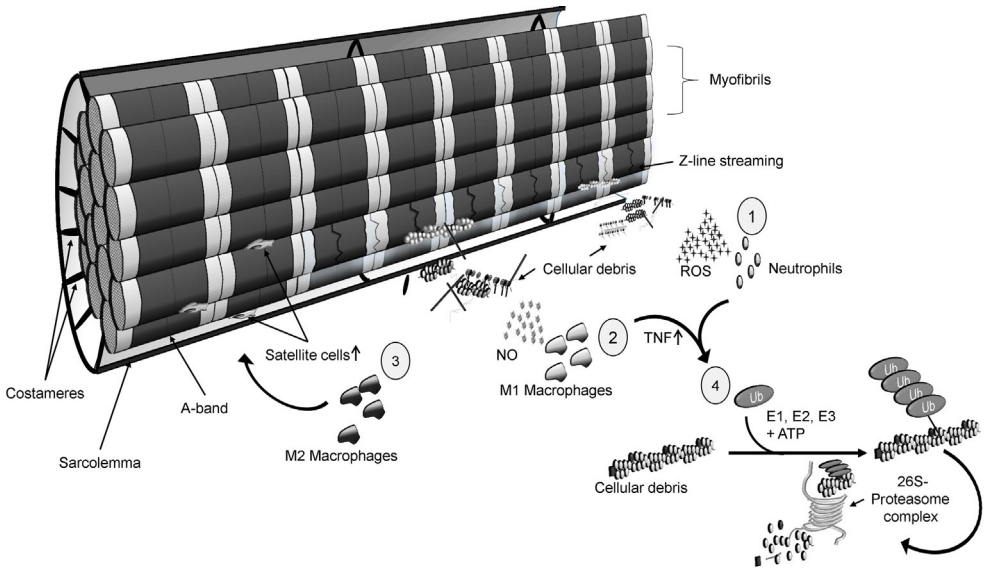

Fig. 17.1 The inflammatory response following exercise-induced muscle damage. Firstly, neutrophils migrate to damaged muscle fibres and produce reactive oxygen species (ROS) to degrade cellular debris (1). Neutrophils are substituted by macrophages within 24 h, with M1 macrophages removing cellular debris by producing cytotoxic levels of nitric oxide (NO) (2). In the latter stage of muscle damage, a shift from M1 to M2 macrophages is associated with the activation of satellite cells and the subsequent regeneration of muscle fibres (3). Neutrophils and macrophages also express tumour necrosis factor (TNF), which activates the ubiquitin-proteasome pathway (4). This pathway regulates proteolysis by attaching ubiquitin polymers (Ub) to cellular debris via three different types of enzymes (E1–E3 ligases). As a result, these ubiquitin tagged proteins will be degraded by the 26S-proteasome complex. *(From Baumert, P., Lake, M. J., Stewart, C. E., Drust, B. & Erskine, R. M. 2016a. Genetic variation and exercise-induced muscle damage: implications for athletic performance, injury and ageing. Eur. J. Appl. Physiol. 116, 1595–1625 with permission).*

17.3 Polymorphisms associated with EIMD

17.3.1 *ACE* I/D

One of the most investigated exercise-related genetic variants is the insertion/deletion (I/D) polymorphism of an Alu repetitive element within intron 16 on chromosome 17 of the *ACE* gene (Gayagay et al., 1998; Montgomery et al., 1998). This *ACE* polymorphism is represented by several data base (dbSNP) entries all tagging this single insertion/deletion polymorphism (e.g., rs4340, rs4646994, rs1799752, and rs13447447). Angiotensin-converting enzyme (encoded by the *ACE* gene) of the renin-angiotensin system converts angiotensin-I to the vasoconstrictive peptide angiotensin-II, which interacts with different receptors in various tissues causing blood vessels to narrow and, therefore, increases blood pressure. The I-allele is predominantly associated with improved performance in atmospheric hypoxia (Montgomery et al., 1998; Hennis et al., 2015), whereas the D-allele is associated with elite power athlete status (Papadimitriou et al., 2016). With regard to EIMD, this polymorphism has been associated individually (Heled et al., 2007; Yamin et al., 2007) and in combination with other SNPs (Del Coso et al., 2017a,d) with the response to EIMD in young physically active individuals. The majority of these studies reported a detrimental EIMD response of the I-allele carriers, such as increased serum CK activity (Yamin et al., 2007). As this gene is predominantly expressed within the lung and adipose tissue, and only at a low level in skeletal muscle (Consortium, 2015), it can be assumed that the genetic variation might indirectly influence the response to EIMD. D-allele carriers are reported to have an elevated blood supply and growth of new blood vessels in response to strenuous exercise (Vaughan et al., 2013) potentially promoted by enhanced *ACE* activity (Rigat et al., 1990; Phillips et al., 1993; Danser et al., 1995). Lower capillary density of I-allele carriers might attenuate neutrophil and macrophage migration to the damaged site and could impair muscle recovery following EIMD. ACE and its receptors are also associated with inflammation, muscle recovery, and pain sensitivity (Song et al., 2014; Dousset et al., 2007; Bedair et al., 2008). The D-allele carriers of the *ACE* SNP may feel less pain and experience improved recovery through the increase of arterial blood pressure due to higher bradykinin degradation (Dendorfer et al., 2001; Murphey et al., 2000).

17.3.2 SNPs of the *IGF2* gene region (e.g., rs3213221 and rs4244808)

Insulin-like growth factor (IGF)-II is produced in several tissues, including skeletal muscle, active immune cells, and potentially the muscle ECM (Ten Broek et al., 2010; Consortium, 2015). It is generally associated with satellite cell differentiation and proliferation (O'Dell and Day, 1998; Stewart et al., 1996) and possibly with ECM integrity (Keller et al., 1999). The genotype-time interaction of SNPs within the *IGF2* gene

(e.g., *IGF2*, rs3213221 and *IGF2-AS*, rs4244808) has been associated individually (Devaney et al., 2007) and in an additive model together with other candidate SNPs (Del Coso et al., 2017a,d) in regard to EIMD. The majority of these SNPs are reported to be associated with immediate strength loss, pain, and serum CK response over time. Increased IGF-II expression might have a beneficial effect on myotube formation (Jiao et al., 2013; Wilson et al., 2003). Although many of the *IGF2* SNPs are associated with immediate strength loss, it can be assumed that the SNPs might not change the IGF-II concentration following an EIMD-intervention *per se*, but IGF-II concentration might be generally different. Chronically elevated IGF-II expression might lead to deposition of the muscle ECM within and surrounding the skeletal muscle, and subsequently to a detrimental muscle structure, as seen in muscular dystrophy (Porter et al., 2002; Haslett et al., 2002). Clearly, the mechanism of this polymorphism merits further investigation, for example whether the paternally expressed *IGF2* gene of this SNP is associated with different responses between male and females (Petry et al., 2011), although this was not the case reported by Devaney et al. (2007).

17.3.3 Interleukin-related SNPs

Cytokines play an important role during the inflammation following EIMD, acting as mediators to either support or attenuate the influx of inflammatory cells to the injury site. They are produced from different tissues including macrophages and even skeletal muscle (Clarkson and Hubal, 2002).

Generally, the proinflammatory interleukin-1β (*IL1B*) cytokine is not significantly affected by EIMD (Mahoney et al., 2008). This is in line with other studies showing that *IL1B* SNPs (e.g., +3954, rs1143634) could not be linked with athletic performance (Cauci et al., 2010). However, individuals with the preferential genotype (rs1143634 CC and rs4848306 TT) of SNPs located within the *IL1B* gene demonstrated an elevated cytokine expression in skeletal muscle 3 days after an acute resistance exercise intervention (Dennis et al., 2004). The expression of this proinflammatory cytokine was amplified when individuals with the abovementioned genotypes also possessed a rare allele within the *IL1RN* gene [C-allele of the T > C (+2018); rs419598 SNP], which encodes the receptor antagonist of IL-1. However, no physiological measures were performed in this study and it cannot be concluded whether these different inflammatory responses within the muscle would have any physiologically relevant outcome.

The cytokine interleukin-6 (encoded by *IL6* on chromosome 7) is a key player in systemic inflammation by mediating other cytokines (Pedersen and Febbraio, 2008). Interleukin-6 is strongly associated with glucose transport in human skeletal muscle (Glund et al., 2007) and with elite power athlete status (Weyerstraß et al., 2018). As EIMD-interventions cause a delayed peak circulatory concentration of interleukin-6 (Fischer, 2006; Toth et al., 2011), this might be an indication of a long-lasting demand

for glucose to remodel the damaged muscle fibers and muscle ECM (Andersen et al., 2011). However, interleukin-6 demonstrates an ambiguous picture and is associated with both a pro-and antiinflammatory responses, depending on the health and age status of the individual. That aligns with the ambiguous patterns of the functional −174 G > C SNP (rs1800795), which is located in the promoter region within the *IL6* gene. The G-allele is linked with an increased IL-6 plasma concentration (Bennermo et al., 2004; Fishman et al., 1998; Pereira et al., 2011) and with a attenuated CK activity response of young healthy individuals following different EIMD investigations (Yamin et al., 2008; Yamin, 2009; Del Coso et al., 2017a,d). However, the G-allele has also been reported to be associated with an increased CK activity response to EIMD in older obese women (Funghetto et al., 2013), while others report no association between this SNP and exertional rhabdomyolysis (Deuster et al., 2013). Therefore, the *IL6* SNP might be not an accurate marker of the EIMD response, particularly between different populations as the underlying mechanism of the *IL6* gene regarding EIMD is not yet fully understood and warrants further investigation.

The proinflammatory cytokine tumor necrosis factor (*TNF*; formerly TNF-α) is mainly expressed in lymphocytes, adipocytes, lung, spleen, and whole blood (GTEx Consortium, 2015). The gene has numerous SNPs, several of which are located in its promoter region affecting gene expression (Nourian et al., 2017). The genotypes of the *TNF* G–308A (rs1800629) SNP were associated with varying serum TNF levels (Cui et al., 2012; Karimi et al., 2009), showing that the TNF expression with respect to the promoter SNPs needs to be considered within the context of the health and age of the individuals studied. The GG genotype of the *TNF* G − 308A (rs1800629) SNP demonstrated a nonsignificant trend individually (Yamin et al., 2008), and statistical significance in an additive model together with other candidate genes (Del Coso et al., 2017a,d) relating to an increased CK activity response hours and days after EIMD. Macrophages of the GG genotype potentially secrete more local TNF after infiltrating the injury site hours after EIMD, attenuating the muscle remodeling process when compared to the A-allele carrier.

17.3.4 SOD2 Ala16Val (C > T; rs4880 or rs1799725)

Mitochondrial superoxide dismutase 2 (*SOD2*) is one of the four investigated genes relating to EIMD, which are predominantly expressed in skeletal muscle and to a lower extent in fibroblasts, liver, kidney, lung, and in adipose cells at baseline (Consortium, 2015). The *SOD2* gene transcript manganese superoxide dismutase, which is an antioxidant protecting cells and mitochondria against reactive oxygen species during inflammation (Huang et al., 2000). The T-allele of the Ala16Val (rs4880 C/T) *SOD2* SNP is linked with impaired enzyme efficiency (Shimoda-Matsubayashi et al., 1996). This might explain the elevated plasma CK values of T-allele carriers following a running

competition (Akimoto et al., 2010) and the relatively low number of athletes carrying the T-allele who perform strenuous sports (that are potentially accompanied by more oxidative stress), compared to controls and athletes from low-intensity and nonmuscle damaging sports (e.g., curling and swimming) (Ahmetov et al., 2014; Ben-Zaken et al., 2013; Weyerstraß et al., 2018). It is assumed that individuals carrying the major C-allele might be better protected against oxidative stress following muscle-damaging exercise.

17.3.5 *SPP1* −66 T > G (rs28357094)

The ECM protein and proinflammatory cytokine, secreted phosphoprotein 1 (*SPP1*; also known as osteopontin), is highly expressed in brain and kidney tissue, and it is also expressed in low amounts in varying tissues including resting skeletal muscle (GTEx Consortium, 2015; Zanotti et al., 2011). However, the rate of expression increases up to 100-times in skeletal muscle following muscle damage in mice (Hoffman et al., 2013; Hirata et al., 2003). SPP1 attracts macrophages (Hirata et al., 2003), and possibly neutrophils (Yang et al., 2014) to the injury site. The minor G-allele of the −66 T > G (rs28357094) SNP, which is located in the promoter region of the SPP1 gene, is linked with reduced *SPP1* expression (Giacopelli et al., 2004; Barfield et al., 2014). Carrying the G-allele is associated with generally elevated upper arm muscle volume in women, but without any change in muscle strength (Hoffman et al., 2013). Further, the G-allele is linked with both a detrimental response to EIMD (resulting in increased muscle swelling, muscle strength loss and CK values) and accelerated disease progression of Duchenne muscular dystrophy (Hoffman et al., 2013; Barfield et al., 2014; Pegoraro et al., 2011). Further investigations revealed that the promoter sequence of the *SPP1* gene includes varying enhancer sequences for several steroid hormone-binding sites (e.g., oestrogen and glucocorticoid receptor). The G-allele leads to an increased expression rate when treated with oestrogen in human myoblasts in vitro (Barfield et al., 2014) potentially explaining some reports of gender specific results following EIMD (Hoffman et al., 2013). However, the low number of participants in the study by Barfield et al. (2014) warrants further investigation to confirm the results.

17.3.6 *SLC30A8* (C > T; rs13266634) SNP

Immune system and macrophage function is dependent on zinc during an inflammatory event. Zinc homeostasis is regulated by the family of metal transporters, such as of the SLC30A8 family, that corresponds to the family of exporters (Gao et al., 2017). The gene of the solute carrier family 30 (zinc transporter) member eight (ZnT8) protein is located on chromosome 8 and it is predominantly expressed in pancreatic islet beta cells (Lemaire et al., 2009; Chimienti et al., 2006). The TT genotype of a SNP (C > T; rs13266634) within the *SLC30A8* gene is reported to be associated with a lower risk of suffering diabetes type 2 (Cheng et al., 2015), and with reduced soreness and plasma CK, as well as

myoglobin levels following EIMD in men (Sprouse et al., 2014). The nonpreferential C-allele might reduce the function of the zinc reporter followed by disturbed macrophage function and insulin production. Lower activity of macrophages and reduced energy supply (Nielsen et al., 2015; Asp et al., 1996) during muscle remodeling might cause the attenuated EIMD response.

17.3.7 *TRIM63* (rs2275950) SNP

The E3 ubiquitin-protein ligase muscle RING-finger protein-1 (MuRF-1), which is encoded by the *TRIM63* gene, is almost solely expressed within skeletal and, to a lesser extent, in cardiac muscle tissue (GTEx Consortium, 2015). The ligase plays an important role in muscle atrophy via the ubiquitin-proteasome pathway (Bodine et al., 2001). A-allele carriers of the *TRIM63* (rs2275950) SNPs have been associated with greater muscle strength and less muscle pain after an EIMD intervention compared to GG homozygotes (Baumert et al., 2017). Increased gene expression of the A-allele and a subsequent increase in degradation activity of the corresponding enzyme after intense exercises might lead to better substitution of damaged proteins and, therefore, to enhanced recovery following EIMD. Further, recent investigations suggest that MuRF-1 interacts with titin at the M-line of the sarcomere (Centner et al., 2001). The *TRIM63* A-allele is potentially linked to a higher affinity for titin's strain sensing kinase domain, which causes greater kinase activation and enables an intrinsically stronger muscle fiber for lateral and longitudinal force transmission. It might therefore be assumed that a stiffer muscle fiber is less susceptible to acute damaging eccentric contractions (Baumert et al., 2017).

17.4 Part II: Consequences of the response to EIMD

Exorbitant strenuous exercise and insufficient recovery of previously fatigued and damaged muscles can lead to two different forms of muscle injury driven by varying underlying mechanisms. Certain individuals may be genetically predisposed to reduced muscle damage and enhanced recovery between training sessions. Indeed, the damage response to eccentric exercise is highly variable and certain gene variants, or polymorphisms, have been associated with exertional rhabdomyolysis (Scalco et al., 2015) and muscle strain injury (Larruskain et al., 2018) (Fig. 17.2).

The first injury type is exertional rhabdomyolysis, which can be caused by sustained exposure to weight bearing exercise (e.g., military training and marathon) involving prolonged muscle fiber contraction (Rawson et al., 2017). This overexertion triggers the release of intracellular calcium and the activation of protein degradation pathways, which can cause necrosis and the release of nephrotoxic muscle proteins such as myoglobin (Greenberg and Arneson, 1967; Clarkson, 2007) and an elevation of creatine kinase activity (Brancaccio et al., 2007) into the blood stream. This can lead to clinically relevant symptoms such as compartment syndrome or renal failure and can even cause death

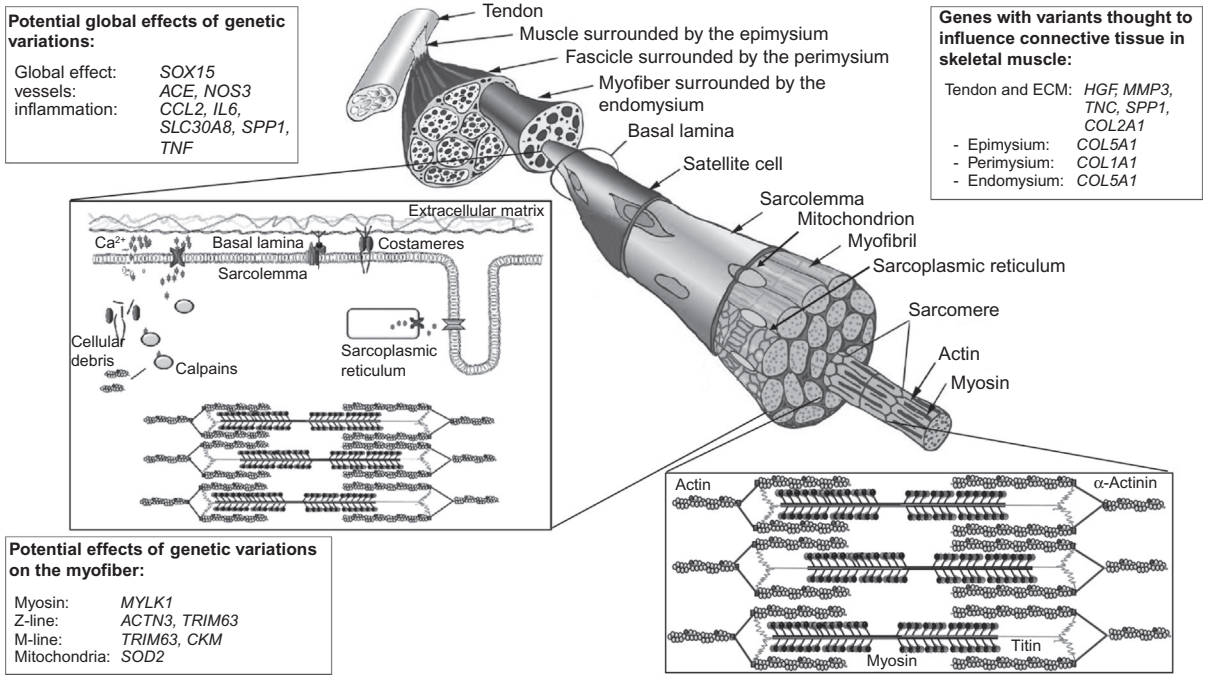

Fig. 17.2 The potential effects of genetic variation on exercise-induced muscle damage, exertional rhabdomyolysis and strain injuries. *ECM*, extracellular matrix; *EIMD*, exercise-induced muscle damage. *(Modified from Zammit, P. S. 2017. Function of the myogenic regulatory factors Myf5, MyoD, myogenin and MRF4 in skeletal muscle, satellite cells and regenerative myogenesis. Semin. Cell Dev. Biol. 72, 19–32 (Elsevier) and Baumert, P., Lake, M. J., Stewart, C. E., Drust, B. & Erskine, R. M. 2016a. Genetic variation and exercise-induced muscle damage: implications for athletic performance, injury and ageing. Eur. J. Appl. Physiol. 116, 1595–1625 with permission).*

(Rawson et al., 2017; Scalco et al., 2015). Therefore, it is not surprising that genetic variations, which are associated with exertional rhabdomyolysis, are largely linked to changes in intracellular calcium release (Scalco et al., 2015). The genetic predisposition to rhabdomyolysis (many of which are rare) has been reviewed previously (Scalco et al., 2015; Rawson et al., 2017) and this chapter focuses on the common relevant polymorphisms associated with strenuous exercise (Deuster et al., 2013).

The second type of injury is muscle strain. Muscle strain injuries can be triggered by exposure to a number of maximal sprints (Malone et al., 2017, 2019; Chumanov et al., 2012; Duhig et al., 2016). A strain occurs when exposure to excessive tensile force leads to overstraining of the myofibers and rupture, usually close to the myotendinous junction (Järvinen et al., 2005). Depending on the severity of the rupture, the required recovery time for muscle strains can vary from a few days to several weeks. Muscle strain injuries carry a high risk of recurrence (Orchard and Best, 2002) and are common in sports such as soccer, where approximately 30% of injuries are muscle strains (Ekstrand et al., 2011a). Consequently, understanding the complex etiology of muscle strains would be advantageous to sports people, sports coaches, and recreational athletes.

Recent investigations suggest that the muscle ECM plays an important role in skeletal muscle strain injury. The muscle ECM consists of three layers with a variety of substances including different types of collagen, glycoproteins (e.g., *TNC*), and proteoglycans (Kjær, 2004). The endomysium (surrounding muscle fibers) and epimysium (surrounding the muscle) might have an important function to store and release elastic energy and to protect the remaining MTU from overstretching (Gillies and Lieber, 2011; Brazile et al., 2017). SNPs which contribute to endo- and epimysium elasticity might attenuate damage within the MTU and reduce injury risk. Further, it is thought that muscle force is predominantly transmitted laterally via the perimysium (surrounding muscle fascicles) to the tendon and ultimately to the bone (Ramaswamy et al., 2011; Hughes et al., 2015). Recent investigations suggest that certain structural damage to the perimysium differentiates between EIMD and muscle strain injury (Balius et al., 2018; Mueller-Wohlfahrt et al., 2013). The role of the muscle ECM following EIMD and injury is supported by the observation that macrophages and monocytes infiltrate the endomysium and especially the perimysium of the injured area of the muscle (Hubal et al., 2008; Paulsen et al., 2010). Furthermore, synthesis of different collagen types (type I, III, and probably IV) within the endomysium and the perimysium increase after contraction-induced damage (Mackey et al., 2004; Koskinen et al., 2001).

17.4.1 *ACTN3* (rs1815739) SNP

Unquestionably, the most investigated common polymorphism in the area of sports genetics is the *ACTN3* (R577X; rs1815739) SNP. The encoded protein is located within the Z-line, which plays an important functional role in muscle force transmission, both

longitudinally and laterally (Monti et al., 1999; Turrina et al., 2013). That is highlighted by the fact that this is the only identified SNP so far which is associated with both exertional rhabdomyolysis (Deuster et al., 2013) and muscle strain injuries (Massidda et al., 2019). The C > T substitution results in a stop codon (X allele), preventing the expression of the amino acid, arginine (hence the term "R-allele" is given to the major allele that encodes arginine) (Nowak et al., 1999) and therefore individuals with the XX genotype are unable to encode the α-actinin-3 protein. While α-actinin-2 is uniformly expressed in all muscle fiber types, α-actinin-3 is exclusively expressed in both fast fiber types, IIa and IIx, in humans (Mills et al., 2001). The R-allele of the nonsense *ACTN3* R577X (rs1815739) SNP is associated with elite athletes of sprint sports (Weyerstraß et al., 2018; Yang et al., 2003). The majority of investigations also link the R-allele with reduced susceptibility to muscle damage (Seto et al., 2011; Vincent et al., 2010), especially in long-lasting, impact-generating endurance events (Belli et al., 2017; Del Coso et al., 2017b,c). Some (Massidda et al., 2019) but not all investigations (Miyamoto et al., 2018; Larruskain et al., 2018 also link the ability to encode ACTN3 with enhanced protection against muscle strain injuries. However, other investigations demonstrate no differences between the *ACTN3* polymorphism (Clarkson et al., 2005), or show a beneficial effect of being an X-allele carrier following EIMD (Venckunas et al., 2012) or in muscle injury risk (Iwao-Koizumi et al., 2014).

It was recently discovered that deficiency of α-actinin-3 results in a shift in fast-twitch fibers toward oxidative metabolism, due to an enhanced activation of calcineurin (Seto et al., 2013). The different metabolic handling between these genotypes lead to significantly higher calcium release in *ACTN3* deficient mice during muscle contractions (Quinlan et al., 2010; Head et al., 2015). The increased calcium release might explain the higher risk in X-allele carriers for exertional rhabdomyolysis following long-lasting weight bearing exercises (Deuster et al., 2013), as theoretically this would further activate protein degradation pathways. Further, deficiency of α-actinin-3 results in the upregulation and accumulation of other Z-line proteins (Seto et al., 2011). This potentially causes decreased stability and rigidity of type II_a fibers of X-allele carriers in general (Broos et al., 2012) and after eccentric contractions (Broos et al., 2019), which might lead to a greater risk of EIMD and muscle strain injuries, against which R-allele carriers are better protected. However, it should be noted that there is more evidence of an effect of the *ACTN3* SNP on the EIMD response than for risk of muscle strain injury.

17.5 Other SNPs associated with exertional rhabdomyolysis

17.5.1 *CKM* NcoI (T > C) (rs8111989)

In addition to the *ACTN3* SNP, there are two other SNPs associated with exertional rhabdomyolysis. High-energy usage is indicated by the decrease of ATP and increase of AMP, leading to the activation of AMP kinase and the increase in cytosolic Ca^{2+}

(Ojuka, 2004). The skeletal muscle creatine kinase (CKM) is bound to the M-Line structure and to the sarcoplasmic reticulum of myofibrils (Wallimann et al., 1992; Brancaccio et al., 2007) and acts to replenish ATP through creatine kinase-mediated phosphotransfer, as well as the reverse reaction. A polymorphism within the *CKM* gene, the Nco1 (T > C) (rs1803285/rs8111989) (Heled et al., 2007; Deuster et al., 2013; Chen et al., 2017) located in the 3′ untranslated region of the chromosome 19 (Nigro et al., 1987; Wilson et al., 1995) has been identified to alter the response following EIMD individually (Heled et al., 2007; Deuster et al., 2013) and in an additive model together with other candidate genes (Del Coso et al., 2017a,d). Intriguingly, the *CKM* gene is located in a similar region of the ryanodine receptor 1 gene (Robinson et al., 2006), which includes several mutations linked to varying forms of rhabdomyolysis (Scalco et al., 2015). Individuals of the Nco1 (rs8111989) CC genotype are reported to have a 3.1 times greater risk of suffering exertional rhabdomyolysis than carriers of the T-allele (Deuster et al., 2013). In contrast to this study, TT genotypes had a sixfold higher risk of being a high responder to circulating CK following an eccentric intervention including stair workouts and squats compared to C-allele carriers (Heled et al., 2007). However, other investigations could not find any significant differences between the genotypes of the Nco1 SNP (Miranda-Vilela et al., 2012; Yamin et al., 2010). The somewhat ambiguous results between studies with varying methodological approaches warrant further investigations. The *CKM* Nco1 (T > C) (rs8111989) SNP might be indirectly linked to intracellular calcium release, where the SNP might alter the enzyme activity of CK to hinder AMP accumulation during strenuous exercise, or to produce ATP for the active restoration of calcium in the intracellular sarcoplasmic reticulum of previously contracted muscles. The genotype, which supports elevated intracellular CK concentration during strenuous exercise, might increase calpain activation and might therefore cause greater protein degradation.

17.5.2 *MYLK*-related SNPs

The third SNP which is related to exertional rhabdomyolysis is located within the *MYLK1* gene (also known as *MLCK*). MYLK1 expresses the enzyme myosin light-chain kinase of smooth muscle cells predominantly within arteries, colon, and the esophagus (GTEx Consortium, 2015; Khapchaev and Shirinsky, 2016). However, *MYLK1* also supports healthy muscle development in the early phase of skeletal muscle cell differentiation (Herring et al., 2000; Wu et al., 2003). The enzyme is activated by Ca^{2+} binding to calmodulin and it phosphorylates the myosin regulatory light chain that improves the interaction with actin filaments during contractions. There are two different SNPs (G49A, rs2700352; C37885A rs28497577) identified in the *MYLK* gene related to EIMD (Clarkson et al., 2005; Del Coso et al., 2017d). The A-allele of the C37885A (rs28497577) *MYLK* SNP is associated with elevated strength loss and higher CK activity

after eccentric exercise (Clarkson et al., 2005) and the A-allele was also associated with a five times higher risk of suffering exertional rhabdomyolysis compared to the CC genotype (Deuster et al., 2013). The GG genotype of the G49A (rs2700352) *MYLK* SNP is associated with increased baseline strength and lower CK activity, as well as myoglobin concentration, 4 days after acute resistance exercise (Clarkson et al., 2005), but no risk for exertional rhabdomyolysis exists between the alleles (Deuster et al., 2013; Scalco et al., 2015). It has recently been found that the G49A GG genotype demonstrated an increased myotube formation following an artificial skeletal muscle injury model in vitro (Baumert, 2019), supporting the effect of *MYLK1* during skeletal muscle differentiation. As this gene is predominantly known to be expressed in smooth but not skeletal muscle cells, further investigation should address whether the *MYLK1* gene has a function following EIMD, for example, as a regulator of skeletal muscle myosin light-chain kinase phosphorylation, sarcomere organization, and skeletal muscle contraction, which might cause higher muscle strain and subsequently greater muscle damage following strenuous exercise.

17.6 Other SNPs associated with muscle strain injuries

17.6.1 SNPs of collagen-encoding genes

The muscle ECM, which comprises mostly collagen, accounts for 1%–10% of muscle mass dry weight (Dransfield, 1977) and is a major contributor to the passive force of skeletal muscles at high strain (Gillies and Lieber, 2011). The MTU comprises different types of collagen, with collagen type I and III being the most abundant types of tendon and muscle collagen (Kjær, 2004). Collagen type I fibrils are stiff structures that are predominantly present in the perimysium and almost equally distributed with type III collagen fibrils in the endomysium and epimysium (Gillies and Lieber, 2011). Collagen type V, a relatively minor collagen of the muscle ECM, potentially interacts with type I and III fibrils and is predominantly present in the endomysium and epimysium of the muscle ECM (Nakamura et al., 2007; Mak et al., 2016). SNPs within the collagen genes might affect the regulation of the collagen network, which might lead to different mechanical MTU properties. For instance, SNPs of collagen-encoding genes encoding the alpha-1 chain of type I (*COL1A1*, *rs1800012*, and *rs2249492*) are associated with differences in maximum muscle strength (Baumert et al., 2018; Van Pottelbergh et al., 2001), but not with EIMD or muscle strain injuries (Pruna et al., 2013). It is possible that other less stiff proteins, present within the different layers of the muscle ECM, might contribute more to the maladaptation to strenuous exercise.

Indeed, the *COL5A1* (rs12722) SNP has been comprehensively investigated with regard to injury risk, and the T-allele has been identified as a risk factor for tendinopathies (Mokone et al., 2006; September et al., 2009) and for anterior cruciate ligament ruptures in females (Posthumus et al., 2009). Regarding skeletal muscle, the major T-allele

homozygotes of the *COL5A1* (rs12722) SNP have been associated with higher muscle soreness and an impaired range of motion (ROM) recovery of following an EIMD intervention (Baumert et al., 2018), and there are preliminary data to show that the T-allele is associated with the severity of skeletal muscle injuries (Massidda et al., 2015; Pruna et al., 2013). The function of the endo- and epimysium might have an important role to store and release elastic energy and to distribute stress over the entire muscle consistently (Brazile et al., 2017). The T-allele is associated with increased messenger RNA (mRNA) stability by increasing the abundance of collagen type V, which was associated with smaller fibril diameter, increased tissue-fibril density, and tissue stiffness (Posthumus et al., 2011). Therefore, the T-allele might contribute to a stiffer tendon/muscle ECM, leading to a better force transmission, that is, performance enhancement in running (Posthumus et al., 2011). However, this muscle ECM/tendon might be more susceptible of being overstretched by extreme external forces, making T-allele carriers more vulnerable to EIMD and to soft tissue injuries compared to the C-allele carriers.

Contrary to this, the minor A-allele of the missense *COL2A1* (rs2070739) SNP revealed higher perceived muscle soreness compared to the G homozygotes after an EIMD-intervention (Baumert et al., 2018). Type II collagen is commonly expressed in cartilage, which is found on many joint surfaces and intervertebral discs (Gustafsson et al., 2003). However, type II collagen is also present in the tendon near the bone insertion (Adamczyk et al., 2008; Buckley et al., 2013), as it forms strong fibrils but which are smaller in diameter compared to type I collagen fibrils in tendon and ligament (Mow and Huiskes, 2005). Further, overtraining caused by chronic downhill running upregulates *COL2A1* expression measured in biopsies of the exercised tendons in rats, indicating a (mal)adaptation to chronic overuse training (Archambault et al., 2007). The *COL2A1* (rs2070739) SNP may change the RNA expression of procollagen type II (Tarpey et al., 2013), which might lead to different stiffness and elastic properties of the tendon. It could be speculated that GG homozygotes with a potentially more elastic patellar tendon might have a greater capacity to store and release elastic energy (Stafilidis and Arampatzis, 2007), while the contractile apparatus of A-allele carriers might need to accept more eccentric work during the eccentric-induced contractions, potentially leading to increased EIMD.

17.6.2 CCL2 SNPs

The chemokine (C–C motif) ligand-2 (*CCL2;* also known as *MCP1*) is a chemokine produced in several tissues including macrophages and satellite cells (Yahiaoui et al., 2008; Consortium, 2015). It can be classified as an exercise factor, as it mediates systemic changes induced by chronic exercise training (Catoire and Kersten, 2015) and it is classically known to attract macrophages to the injury site to provide an adequate inflammatory response following muscle damage (Lu et al., 2011). In recent years, *CCL2* has been shown to be

involved in the attraction of fibroblasts to the injury site of different tissues via *CCR2*, and with a subsequent upregulation of collagen type I expression through distinct chemokine/chemokine receptor systems in different cell types in vitro (Hara et al., 2013; Moore et al., 2005). Most of the *CCL2* SNPs (i.e., rs1024611, rs2857656, rs4586, rs13900) are related with baseline strength differences (Hubal et al., 2010; Harmon et al., 2010). However, some SNPs, such as the *CCL2* (rs3917878) and its receptor chemokine receptor 2 (*CCR2;* rs3918358), are also associated with delayed muscle strength recovery and *CCR2* T > C (rs1799865) SNP with elevated soreness following EIMD (Hubal et al., 2010). Several publications revealed that the GG genotype of the *CCL2* G > C (rs2857656) SNP is associated with a higher baseline muscle strength (Harmon et al., 2010), but with a higher severe muscle injuries (Pruna et al., 2013), which could be caused by a higher *CCL2* gene expression (He et al., 2017). However, this SNP did not show any differences between genotypes in the response to EIMD (Hubal et al., 2010). It could be speculated that the alleles of the *CCL2* and *CCR2 SNPs*, which are associated with a higher RNA expression following intense exercise, subsequently attract more fibroblasts to the damaged areas of the muscle ECM. Recurrent strength training events might improve the integrity of the muscle ECM over time. A higher integrity of the muscle ECM might improve the stiffness, which would support an improved transmission of muscle strength from the muscle to the bone of those harboring the preferential alleles in relation to muscle strength. However, a stiffer ECM caused by these alleles might be more prone to be damaged during high-speed eccentric contractions potentially explaining the higher severe of muscle injuries.

17.6.3 *HIF1A* rs11549465

The transcription factor hypoxia-inducible factor-1α (HIF1A) regulates a number of genes in response to hypoxia to stimulate glycolytic metabolism and angiogenesis (Airley and Mobasheri, 2007). Furthermore, HIF1A is an important component of muscle ECM remodeling and skeletal myogenesis, which can also be initiated by mechanical loading (Petersen et al., 2004). The functional rs11549465 SNP is a C > T substitution and is one of at least six polymorphisms identified within the *HIF1A* gene. The rare Ser582 (T) allele has been associated with increased transcription and stability of the HIF1 protein in comparison to the ancestral Pro582 (C) allele (Tanimoto et al., 2003). The majority of research around the rs11549465 SNP to date involves various health conditions, though links to angiogenesis and glycolytic metabolism make *HIF1A* SNPs relevant candidates for exercise-related phenotypes.

Professional soccer players with the CT genotype were found to have lower risk for hamstring muscle injury than CC homozygotes (Larruskain et al., 2018), suggesting that the T allele offers a protective benefit against injury. If transcription and stability of the HIF1 protein is enhanced by the T-allele (Tanimoto et al., 2003), this could partially

explain why CT heterozygotes possess significantly more type IIx muscle fibers than CC homozygotes (Ahmetov et al., 2008). Indeed, HIF1A mRNA and protein levels are reportedly higher in type IIx fibers (Pisani and Dechesne, 2005) and there is an overrepresentation of the T-allele amongst strength athletes compared to nonathletic controls (Gabbasov et al., 2013). Considering these reports, the reduced risk for hamstring injury in T-allele carrying soccer players suggests that a greater proportion of type IIx fibers may offer a protective benefit against hamstring injury. However, it is important to note that easily fatiguing, fast glycolytic fibers demonstrate greater propensity for muscle damage (Lieber and Friden, 1988), thus a greater number of type II fibers may in fact elevate the risk of strain injury, as opposed to offering protection. Nevertheless, collectively possessing a greater number of stronger type II muscle fibers might protect vulnerable fibers from strain injury.

The rs11549465 T-allele increases the risk of hamstring injury requires confirmation in additional populations, though a fiber-type-mediated protective mechanism remains possible if athletes possessing the T allele also have more type IIx fibers. Investigation of this variant with other exercise-related phenotypes and forms of muscle injury is warranted, considering the limited literature available at present.

17.6.4 HGF rs5745678, rs5745697, and rs1011964

Hepatocyte growth factor (HGF) plays a role in the regulation of satellite cell proliferation and differentiation (Gal-Levi et al., 1998) and is encoded by the *HGF* gene. Active HGF is present within the muscle ECM (Tatsumi and Allen, 2004) and the receptor for HGF is Met, with HGF/Met signaling required for migration of satellite cells to an injured site within skeletal muscle (Webster and Fan, 2013). Regulating the expression of HGF and/or Met during muscle regeneration is suggested as a key mechanism in the control of HGF/Met signaling during the process of cell differentiation and regeneration (Gal-Levi et al., 1998). Due to the specific role of HGF in response to muscle injury, it could be considered an appropriate candidate gene for association with sports injury.

HGF is elevated both in the circulation and within the skeletal muscle after an EIMD intervention, potentially supporting the migration of the satellite cells to the injury side (O'Reilly et al., 2008). However, to our knowledge, there is no report regarding any *HGF* SNP and EIMD, and only one study has investigated a link between SNPs of the HGF gene and sports injury (Pruna et al., 2017). In this investigation, associations were described between three separate HGF SNPs and noncontact muscle injury in a cohort of 73 elite soccer players. Specifically, the rs5745697 G > T and the rs1011694 A > T SNPs were associated with the incidence of injuries. Players carrying the GG genotype of the rs5745697 SNP had a lower injury rate than T allele carriers (8.4 vs. 12.3/1000 h), while those homozygous for the rs1011694 A allele demonstrated a lower rate of injury compared to T allele carriers (8.4 vs. 12.08/1000 h). Furthermore, two

SNPs were associated with the recovery from muscle injuries. Specifically, players with the rs5745678 A allele had a mean absence of 19.8 compared to the 27.7 days of the TT genotype, while players carrying the C allele of the rs5745697 having a mean absence of 20.7 days as opposed to 27.5 in TT homozygotes. Therefore, the rs5745697 SNP was the only one of the three SNPs to associate with both injury rate and recovery time, with the rs5745678 and rs1011694 variants being separately linked to injury rate and recovery time, respectively.

At present there is little mechanistic explanation for the associations described by Pruna et al. (2017). Those authors suggested those with the wild-type genotype for each *HGF* SNP benefit from a "normal" interaction between HGF and Met, ensuring no elevated injury rate or absence. Pruna and colleagues cite previous research to suggest that an established interaction between HGF and its receptor allows the correct signaling cascade, resulting in cell proliferation and migration, and that absence or reduction in receptor activity leads to abnormal skeletal muscle formation (Gutiérrez et al., 2014). Further, HGF seems to play an important role regarding the interaction between the ECM and myoblast migration (González et al., 2017). However, the exact mechanism by which these *HGF* SNPs might influence muscular injury in soccer players remains unclear. Future work should seek to confirm these associations in similar populations, and to investigate the molecular consequences of these and other *HGF* SNPs.

17.6.5 MMP3 rs679620

Matrix metalloproteinases (MMP) have an important role in maintaining the functional integrity of myofibers by degrading components of the ECM (e.g., collagen), as well as regulating the migration, differentiation and regeneration of skeletal muscle cells (Goetsch et al., 2003; Davis et al., 2013; Chen and Li, 2009). Variations in MMP expression are linked with several diseases, such as muscle pathologies (Choi and Dalakas, 2000), and they are thought to alter the function of the ECM and influence the rate of muscle regeneration (Goetsch et al., 2013; Carmeli et al., 2004). The rs679620 SNP of the *MMP3* gene is a missense mutation characterized by a T > C substitution with high linkage disequilibrium to the rs3025058 variant, a functional polymorphism located within the gene's promoter region (Ye, 2006). The C-allele of the *MMP3* (rs679620) SNP is thought to decrease *MMP3* transcription (Foster et al., 2012), as it is in high linkage disequilibrium with the 6A-allele of the *MMP3* (rs3025058) SNP (Ye, 2006; Taylor et al., 2008). However, the promoter activity of the alleles seem to be population and tissue dependent (Zhang, 2018) and there is no clear picture as to whether the rs679620 T > C SNP substitution results in increased or decreased MMP3 expression (Ye, 2006; Medley et al., 2003; Chen et al., 2012). The limited studies of the rs679620 variant and injury risk primarily relate to tendons (Raleigh et al., 2009; El Khoury et al., 2016), though a role in muscular injury has been considered.

The conclusions which emerge from investigations of the rs679620 variant in relation to sports injury are conflicting. In observations from our laboratory (Baumert, 2019), A-allele carriers demonstrated a faster strength recovery following EIMD potentially due to increased *MMP3* expression, and therefore, increased degradation activity of this enzyme. This mechanism might results in an increased muscle ECM integrity and to better force transmission from the muscle over the tendon to the bone in the long term. However, Pruna et al. (2017) found no association with noncontact muscle injury in 74 elite soccer players when 220 separate muscle injuries were recorded over five soccer seasons, despite demonstrating associations with other SNPs. Conversely, a study in another elite soccer cohort reported an association between the rs679620 SNP and hamstring injury (Larruskain et al., 2018), where 160 hamstring injuries were recorded from 107 players. Associations were reported, linking the rs679620 SNP to acute, overuse, severe, and recurrent hamstring injuries, when combined and with each variable analyzed separately. Compared to the CC genotype, each T allele doubled the risk of hamstring injury, and of seven different SNPs associated with hamstring injury, the *MMP3* rs679620 revealed the strongest statistical association. If, as suggested previously, the T allele increases *MMP3* expression (Medley et al., 2003), the findings of Larruskain et al. (2018) could be interpreted to suggest that elevated *MMP3* expression, through the presence of both T alleles, could double hamstring injury risk. However, to our knowledge, these are the only existing studies concerning sports-related muscle injury and *MMP3* SNPs, and suggested mechanisms for the reported associations remain speculative.

Expression of *MMP3* affects collagen degradation and might influence the recovery of skeletal muscle, because type-I collagen deposition is abundant in the ECM surrounding myofibers following strenuous injury (Goetsch et al., 2003). The correct balance between collagen deposition and degradation is key to scar tissue formation (Chen and Li, 2009) and contributes to muscular stiffness (Feit et al., 1989), which purportedly increases tolerance to subsequent injury (Lapier et al., 1995). If *MMP3* expression rises above normal physiological levels and increases collagen degradation, vital postinjury collagen deposits around myofibers could be degraded and inhibit tolerance to subsequent exercise bouts (Takagi et al., 2016). This mechanism might explain the association with hamstring injury documented by Larruskain et al. (2018), particularly the finding that each T allele, which is associated with elevated *MMP3* expression, doubled the risk of injury. Indeed, elevations in *MMP3* expression could explain the association between the rs679620 T allele and severe, overuse and recurrent hamstring injuries, as each indicates a compromised postinjury healing response. It is important to reiterate no association was detected in an earlier study (Pruna et al., 2013) and to remain cognizant of previous associations between the rs679620 variant and tendon injury, as the biceps femoris long head (the most commonly injured muscle in a HSI (Ekstrand et al., 2011b)) contains an intramuscular aponeurosis extending into the muscle belly which is sometimes torn in

hamstring strains (Brukner and Connell, 2016). The influence of tendon properties on injury to other skeletal muscles should also not be overlooked. For example, Hicks et al. (2013) found that young healthy men had stiffer patellar tendons than young healthy women, and that men experienced more fascicular lengthening than women during maximum eccentric contractions. A stiffer tendon may therefore cause more mechanical damage during lengthening contractions, thus SNPs that influence tendon properties may indirectly influence the response to EIMD and muscle injury risk. Further evidence concerning *MMP3* SNPs and muscle injury is required before considering the gene as a marker of injury risk.

17.6.6 *NOS3* (rs1799983 and rs2070744) SNP

Nitric oxide (NO) exerts numerous biological functions, which influence mitochondrial respiration, muscle contractility, and skeletal muscle repair following injury (Stamler and Meissner, 2001). Further, NO is a strong vasodilator, which is important for blood supply to skeletal muscle (Heydemann and McNally, 2009) and is strongly associated with glucose uptake in skeletal muscle during exercise (McConell and Kingwell, 2006; Kellogg et al., 2017). Nitric oxide synthase-3 (NOS3) is the rate-limiting enzyme involved with NO production. Within the *NOS3* gene, which encodes the enzyme, two SNPs have been identified, that is, rs1799983 and rs2070744.

The rs1799983 SNP is characterized by a G > T substitution and while the kinetics of nitric oxide synthesis are believed to be the same for each allele, the T allele appears more vulnerable to proteolytic cleavage compared to the G allele (Tesauro et al., 2000). Consequently, this could lead to increased NOS3 activity and greater nitric oxide production in G allele carriers, where less of the NOS3 protein's peptide bonds are broken through proteolytic cleavage compared to T allele carriers. In the previously mentioned study by Larruskain et al. (2018), the rs1799983 SNP was associated with hamstring injury among 107 elite soccer players. Specifically, the G allele was associated with 1.35 times the risk of hamstring injury. The mechanisms by which the rs1799983 SNP might influence the risk of muscle injury are unclear, and the number of studies investigating this variant remains modest. Previous studies using animal muscle or C2C12 cells show that NOS3 plays an important role in skeletal muscle cell differentiation and fusion (Long et al., 2006; Sellman et al., 2006). However, Sessa et al. (2011) suggest the G allele may be beneficial to athletes competing in power-based sports. The findings that the G allele benefits power-based athletes, yet also appears to increase the risk of hamstring injury, are somewhat paradoxical as elite power athletes would be expected to benefit from robustness against injury in order to reach and to compete at a high level. However, power-based athletes might be expected to possess a greater proportion of type II muscle fibers, and it is type II muscle fibers that are most commonly subjected to strain injury (Macaluso et al., 2012). It could therefore be suggested that more strain injuries would be evident in

athletes with a greater proportion of type II fibers. It may be of interest to investigate whether greater vulnerability for proteolytic cleavage in T allele carriers has any influence on injury risk, particularly as T allele carriers appear to have the lowest risk of hamstring injury when considering the study by Larruskain et al. (2018).

17.6.7 TNC rs2014772

Tenascin-C (TNC) is a pro-angiogenic ECM protein expressed in regenerating myofibers and at the myotendinous junction as a response to mechanical loading (Flück et al., 2008). TNC assumes an important role in the muscle damage-repair cycle, providing strength and elasticity in order to resist mechanical force, and as a glycoprotein, it is tasked with regulating cell-matrix interactions (Flück et al., 2008). Expression of TNC is enhanced following eccentric contractile activity (Crameri et al., 2004) and acts to assist myogenesis, wound healing and angiogenesis to repair damaged muscle (Flück et al., 2008). The rs2104772 SNP of the *TNC* gene is a T > A substitution, with a leucine to isoleucine substitution proposed to contribute to structural instability in the fibronectin type III-D domain of TNC (Matsuda et al., 2005). With a large number of sports-related muscle injuries occurring close to the myotendinous junction (Kalimo et al., 1997), expression of structural proteins such as TNC could affect this region's response to mechanical load and consequently to injury risk.

The rs2104772 SNP of *TNC* is a suitable candidate for investigating both muscle and tendon injuries, but is not associated with hamstring muscle injury in a study of 73 elite male soccer players over five seasons (Pruna et al., 2013). Nevertheless, a subsequent study in a separate population found each rs2104772 A allele to be associated with a 1.65 times greater risk of hamstring injury (Larruskain et al., 2018). It is unclear why discrepancies exist between these studies, though both would benefit from greater sample sizes, which is understandably challenging within an elite sport setting. Interestingly, the rs2104772 A allele has been associated with increased risk for Achilles tendinopathy (Saunders et al., 2013) and contributes to a haplotype associated with poor rotator cuff injury healing (Kluger et al., 2017). Despite few associations with muscle injury to date, the aforementioned associations in other tissues suggest a possible relationship with injury risk in the muscle-tendon unit. It could be that SNPs influencing tendon tissue could affect the injury risk at (or close to) the myotendinous junction, especially as many muscle strain injuries occur within this region. Indeed, hamstring strain is one such injury and is one of the most common injuries in team sports (Opar et al., 2012).

Conclusive evidence surrounding the mechanistic consequences of *TNC* SNPs, such as rs2104772, and their link to sports-related muscle injury are lacking. While associations with muscle injury are evident in one study, replicative results are required before variants of the *TNC* gene can be considered as potential markers relating to injury risk.

17.6.8 SOX15 rs4227

The SRY-related HMG-box (Sox15) protein encoded by the *SOX15* gene is postulated to assume a critical role in myogenic differentiation, determining the fate of skeletal muscle cells during development (Lee et al., 2004). A small number of *SOX15* polymorphisms have been identified, though whether these influence skeletal muscle function or muscle injury is unclear.

The rs4227 SNP involves a G>T substitution, where the T allele represents the ancestral allele and the G is the minor allele. The functional and molecular consequences of this substitution are unclear. Nevertheless, Pruna et al. (2017) included a higher number of muscle injuries and SNPs studied in their earlier work (Pruna et al., 2013). One of these SNPs was the *SOX15* rs4227 SNP. An association was reported between the rs4227 SNP and muscle injury incidence, expressed per 1000 h of soccer exposure (training and match play). Those homozygous for the ancestral T allele (TT genotype) had the lowest injury incidence (7.8 injuries per 1000 h) compared to carriers of the minor G allele (10.2/1000 h and 14.8/1000 h for the GT and GG genotypes, respectively). While a mechanistic explanation remains unclear, Pruna et al. propose their results as an indication that the T allele is required for normal formation of skeletal muscle, and that those with the GG genotype have greater injury rates due to a negative effect on SOX function. While this theory is based on the suggestion that incorrect function (or inactivation) of SOX15 decreases cell proliferation and perturbs muscle regeneration, based on previous work by Meeson et al. (2007), a great deal of mechanistic work is needed to test the hypothesis. Although the skeletal muscle of SOX15 knockout mice was found to exhibit normal skeletal muscle development, cross-sectional images of the skeletal muscle indicated a less pronounced ECM of SOX15 deficient mice (Lee et al., 2004). Given that many other Sox factors interact with the ECM (e.g., SOX9 regulate collagen type II expression) (Bell et al., 1997; Guilak et al., 2009), whether there is an association between SOX15 and the development of the ECM requires further investigation. In the meantime, future candidate gene studies should seek to investigate the association with muscle injury, in order to justify mechanistic investigation of the effects of the rs4227 SNP on skeletal muscle.

17.7 Summary and conclusion

This chapter highlights the genetic variations that have been associated with the variable response to EIMD and/or the risk of muscle injury. Identifying which gene variants are associated with the acute response to EIMD and/or muscle injury risk can highlight potential molecular/cellular mechanisms that may explain why some people appear to be less robust than others in response to acute/chronic strenuous exercise. To conclude, SNPs associated with exertional rhabdomyolysis are predominantly associated

with changes of intracellular calcium release, suggesting a metabolic stress to the muscle structure. Functional SNPs associated with muscle strain injury risk are notably linked with the cytoskeleton as well as the muscle ECM, indicating that extensive mechanical stress/damage tears the stiff structures of the skeletal muscle, and that the muscle ECM may have a causal role in the etiology of muscle strain injuries. SNPs that alter calcium homoeostasis and the integrity of these mechanically stiff muscle structures could subsequently lead to a higher risk of exertional rhabdomyolysis and muscle strain injury, respectively. Large-scale genome-wide association studies are needed for a better understanding of the genetic association with EIMD, exertional rhabdomyolysis, and muscle strain injuries. As there are some indications that the perimysium plays an important role in muscle injuries, further studies should investigate SNPs that are linked specifically to other perimysium-related genes, such as proteoglycans (e.g., decorin and biglycan), different collagen types (e.g., type XII and XIV), and proteins involved in the ECM-muscle interface.

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SECTION V

Nutrigenetics, pharmacogenetics and metabolomics in sport and exercise

CHAPTER EIGHTEEN

Personalized sports nutrition: Role of nutrients in athletic performance

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18.1 Introduction to personalized nutrition: "One size does not fit all"

Since ancient times, nutrition has always been considered an essential condition for maintaining good health. Hippocrates of Kos, the father of medicine, said in 460 BC: "Let food be thy medicine and medicine be thy food." His observations led to associative evidence between diet and health by highlighting how food is able to interfere with our body's physiology by not only acting as an energy provider, but as a modulator of the health/disease balance in a different way for each individual (Tsiompanou and Marketos, 2013) depending on the personal characteristics. Somehow it can be considered a precursor of modern nutritional genomics.

It is fundamental to consider that although nutrients act by modulating some physiologic functions in a dose-dependent manner, each individual responds differently depending on its genotypic and phenotypic characteristics (Ferguson et al., 2016). Therefore, the recommended daily doses suggested by the international nutrition guidelines, based on studies on large populations rather than specific genotypes or phenotypes, should be used with enough flexibility to account for the plethora of genetic, epigenetic, and environmental factors contributing to health and disease in each individual.

Nutritional genomics is a new branch of nutritional medicine based on the concepts of functional genomics and personalized medicine. Empowering the individual biochemical data with genomic data allows the customization of a specific diet for each individual, based on the genotypic characteristics and the nutrients modulatory action on gene expression (Camp and Trujillo, 2014).

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18.1.1 Gene-nutrient interactions: The era of nutrigenetics and nutrigenomics

In the past decade, growing evidence that molecular components in macro- and micronutrients act as potential dietary signals influencing the metabolic status of cells has been collected. Further, the link between genetic polymorphisms in key steps controlling metabolic pathways and the development of chronic diseases such as cardio-metabolic and gastrointestinal diseases has been elucidated. The use of -omics techniques is now allowing to analyze with a systemic approach the response of the whole body to nutrients. Genomic, proteomic, and metabolomic analyses can be used to identify individual molecular characteristics relevant to the definition of a personalized nutritional regime for each individual, depending on the beneficial or negative effect of dietary factors (Müller and Kersten, 2003).

At the genetic level, two terms, nutrigenetics and nutrigenomics, have been developed to indicate subfields focused on the intricate relationships between nutrients, genes, and biological systems.

Nutrigenetics aims to analyze and understand how our genetic background with individual genetic polymorphisms can modulate nutrients absorption, distribution, metabolism, and elimination (ADME), thus affecting response to diet.

Nutrigenomics focuses on the individual sensitivity to nutrients in terms of influence on gene and protein expression and, subsequently, metabolite production, thus providing actionable information on the effects of diet and allowing effective dietary-intervention strategies to prevent diet-related diseases (Mutch et al., 2005).

These two terms are strictly related and should be considered as a single entity when applied to clinical nutritional analyses. Today, genomic tools, such as quantitative techniques (RT-PCR) and high-density microarray and other -omics techniques, equipped with advanced systems biology-oriented informatics can be used to create nutrient-gene interaction networks to analyze for each individual the influence of each nutrient on the entire organism, thus achieving a weighed nutritional regime for wellness maintenance.

18.1.2 Could nutritional genomics be applied to personalized sports nutrition?

One of the most effective applications of nutritional genomics can be in athletic performance. Nowadays every athlete playing sports such as bodybuilding, running, soccer, etc., must conjugate physical activity with a personalized nutritional regime in order to maximize muscle growth and athletic performances. The genetic variability between individuals can affect muscle strength, skeletal structure, heart and lung size, tendon elasticity, etc., leading to different human phenotypes, ultimately influencing sports performance.

Genetic analysis demonstrates that the chances of an individual to have an optimal sporting genotype are lower than 1 in 20 million and with the increase of polymorphisms the score decreases correspondingly (Puthucheary et al., 2011).

Basal muscle mass and response to training are two main factors contributing to muscular performance. Genetic factors account for about 50%–80% of interindividual variation in body mass and this has an important impact on muscular growth response (Puthucheary et al., 2011). Moreover, endocrine functions, muscle fibers composition, psychological aspects, and nutrition can have genotype-associated differences and influence athletic performance.

In particular, influences between genes and nutrients can affect the amount and the type of nutrients ingested with food and, subsequently, bodily functions.

Despite genetic testing for predicting sports performance and talent identification being continuously on the rise in the market, nutrigenetic and nutrigenomic analysis are less known and applied because of the complexity of the interpretation of the functional roles of different polymorphisms in nutrition, especially because every polymorphism can affect directly or indirectly different other genes, proteins or metabolic pathways. More research is needed to clarify the complex gene-nutrient associations' network that could be then used to determine which types of nutrients should be integrated for each individual, and which are not necessary or potentially dangerous.

18.2 Genes, nutrients, and nutraceuticals in athletic performance

18.2.1 Proteins and carbohydrates

Amount and type of proteins and carbohydrates in a personalized nutritional regime are crucial for muscle growth and sports performance.

In recent years significant progress has been made in describing the mechanisms regulating the complex pathways coupling gene expression and protein synthesis and how genetic variability can influence them by starting from the absorption of key nutrients necessary to activate these processes.

Protein intake and amino acids absorption and metabolism are crucial for muscle growth. Genetic differences can affect the amounts of bioactive peptides derived from protein sources and consequently the use of them for muscle activity and growth. Different foods have different protein qualities in terms of the amount of limiting amino acids. Leucine, for example, is a key factor for protein synthesis and enhances the activity of key kinases that regulate the beginning of translation processes such as the mTOR signaling pathway. Genetic polymorphism in LAT1 and LAT2 gene which encode for BCAA amino acid transporters could influence the absorption rate of leucine after ingestion contributing to reduce the amount of leucine available for protein synthesis (Kühne et al., 2007). Further, more than 20 *mTOR* single nucleotide polymorphisms (SNPs) have been identified that contribute to cell overgrowth and cancer development, with seven highly correlated SNPs (rs2536, rs2295080, rs1883965, rs1034528, rs17036508, rs3806317, and rs1064261) (Zining et al., 2016; Wang and Proud, 2006).

The hyperfunctionality of the mTOR pathways due to genetic polymorphisms have an impact on muscle growth and athlete performance in terms of nutrients absorption and protein synthesis. Based on this genetic evidence nutritional strategies concerning the amount of carbohydrates and protein ingested with diet and supplements have to be correctly performed.

In 2009, the pan-European study DiOGenes (diet, obesity, and genes) analyzed the impact of amount and qualities of proteins in weight management in five different diets, measuring in 773 individuals more than 30 plasma proteins such as vascular factors, adipokines, insulin and related hormones, growth factors, satiety hormones, etc. The diet with a moderate increase in protein intake and a modest reduction in the glycemic index of food was related to weight loss. Interestingly, the angiotensin-converting enzyme (ACE) was the one having the most relevance to body weight. In particular, the reduction in ACE due to this diet was related to weight loss in 8 weeks. It is not clear whether ACE-related body weight regulation and dietary protein are strictly related, but numerous genetic polymorphisms in the ACE gene have been revealed to influence the association of circulating level and activity of the ACE protein and weight management. In sports nutrition, people carrying a mutation in the ACE gene are highly recommend to adopt a correct diet regime to prevent fat mass accumulation (Chou et al., 2012).

In terms of body weight management and sports performance, proteins have proved to be a perfect ally. Protein intake has been shown to modify the association between genetic variation in FTO (obesity-associated gene) and body weight measurements in some ethnocultural groups. Higher protein intake (>18% of total daily caloric intake) protects against the obesogenic effects of some FTO genotypes and lead to better individual metabolic profiles. The benefits of high-protein weight management diets have previously been demonstrated, and the results have further suggested a link between FTO, protein intake, and body weight. Clarifying the mechanism that regulates this gene-diet interaction is a clear direction for future research to improve lean muscle mass for bodybuilders among others (Merritt et al., 2018; Jefferson and Kimball, 2001; Muñoz et al., 2017).

Another important aspect in sports nutrition for muscle structure and function is the amount of key amino acids required for collagen synthesis which is a crucial protein in tendon and bone and is also found in both the epimysium and perimysium of skeletal muscle where it acts as a supportive structure in muscle tissue. Collagen synthesis requires proline, lysine, and vitamin C. People carrying polymorphic binding site of the Sp1 transcription factor in the gene (COL1A1, COL1A2) encoding the alpha chain of type I collagen, which are associated with lower grip and biceps strength (Van Pottelbergh et al., 2001), have to increase the daily intake of lysine, proline, and vitamin C through diet or high-quality supplements.

Today, we know that protein and carbohydrates have to be co-ingested to stimulate protein synthesis and muscle growth. In particular, insulin pathways are crucial for muscle

hypertrophy. At the same time, insulin polymorphisms can affect the rate of glucose absorption and muscle hypertrophy. Insulin-like growth factor-I (IGF-I) plays a key role in exercise-associated muscle growth and development. The regulatory region of the promoter of the IGF-I gene is labile. Reports in the literature have suggested that the IGF-I protein plays a major role in strength training (ST)-induced skeletal muscle hypertrophy and strength improvements. A microsatellite repeat in the promoter region of the IGF1 gene has been associated with IGF-I blood levels and phenotypes linked to IGF-I in adult. Kosket et al. demonstrated that IGF1 promoter polymorphism may influence the strength response to ST (Kostek et al., 2005).

Another work demonstrated that the genetic variation C-1245T (rs35767) in the promoter region of the IGF-I gene was associated with higher circulating IGF-I levels, and possibly with increased muscle mass in athletic and nonathletic Israeli populations. The study suggested a contribution for the relatively rare IGF-I TT genotype to endurance performance, and in particular to power sports excellence in Israeli athletes (Ben-Zaken et al., 2013). Different polymorphisms affect the IGF-1 pathway and modulating the amount and the time of carbohydrates ingestion with food during the day seems to be a promising strategy to improve sports performance. It can be speculated that individuals carrying IGF-1 polymorphisms with reduced IGF-1 pathway functionality have to integrate with more whole-grain carbohydrates and high-quality proteins to induce protein synthesis and muscle and brain performances.

Carbohydrates and protein intake with food or supplements is a widely used strategy for muscular recovery and protein synthesis. Genetic and biochemical studies demonstrate that different carbohydrates have different metabolic responses. Fructose ingestion, for example, has been shown to suppress the stimulation of the exercise-induced glucose transporter (GLUT4) in rat skeletal muscle, impairing the expression of genes involved in "remodeling"/postexercise muscle growth; fructose intake alters the expression of genes involved in oxidative metabolism, mitochondrial biogenesis, and proteolytic genes in skeletal muscle. This negative effect of fructose ingestion has been observed for most of these genes both in sedentary people and in those who do exercise, and besides altering the adaptive response of GLUT4, it has also been shown that ingestion of fructose could compromise skeletal muscle responses to exercise by modulating glycogen storage and mitochondrial biogenesis by altering PGC-1α, FNDC5, NR4A3, GLUT4, Atg9, Lamp2, Ctsl, Murf-1, and MAFBx/Atrogin-1 in the skeletal muscles of both sedentary and exercised animals. These results highlight how fructose could compromise the expression of genes involved in metabolic adaptation of skeletal muscles. High fructose intake with diet or supplement may impair skeletal muscle response to exercise, thus limiting sports performance in athletes (Pereira et al., 2017).

On the other hand, long-term glucose uptake contributes to the development of obesity and type 2 diabetes mellitus (T2DM). Obese people tend to eat foods containing glucose, which can lead to glucose dependence, increased glucose in the blood and intestinal

lumen, and exposure of intestinal enterocytes to a high level of glucose in the diet. Recent studies have documented a role for enterocytes in glucose detection. One study was conducted aimed to identify the relevant target genes and the molecular pathways regulated by the high amount of glucose in a well-established in vitro epithelial cell culture model of the human intestinal system (Caco-2 cells). Data on microarray gene expression showed that 679 genes were altered (297 upregulated and 382 downregulated) with high glycemic treatment. The results provide valuable information regarding the molecular and genetic aspects in response of enterocytes to high levels of glucose, which is the main nutritional contributor to the development of obesity and T2DM (type 2 diabetes) (Boztepe and Gulec, 2018).

Studies on bowel and obesity have largely focused on intestinal glycemic detection and reduction of intestinal glucose uptake through delayed carbohydrate breakdown in enterocytes, while the mechanisms by which enterocytes respond to high levels of glucose had not been previously studied. Thus, the identification of candidate genes (e.g., TXNIP) involved in intestinal glucose control for absorption during high glucose uptake adds a new piece of information. In addition, enterocytes exposed to high blood sugar may communicate, possibly via LCN15 (lipocalin 15), with endocrine cells in the gut or in peripheral tissues. This work demonstrates glucose-dependent genetic regulation in enterocytes and has identified important target genes that will facilitate further investigations into the control of glucose metabolism in enterocytes during high glucose uptake (Boztepe and Gulec, 2018; Muñoz et al., 2017).

Goda (2000) showed the molecular mechanism of nutrient intake in modulating intestinal gene expression, cloning 5′ flanking regions of two disaccharidase genes, namely sucrase-isomaltase (SI) and lactase-hydrochloride hydrolase (LPH). Oral feeding of a sucrose-containing diet in rats increases SI mRNA and LPH mRNA levels within 3 h. Among the monosaccharides tested, fructose gave rise to more important mRNA levels of SI and LPH genes, which were accompanied by a coordinated increase in the mRNA levels of two microvillar hexose transporters (SGLT1 and GLUT5). It was shown that fructose, but not glucose, increased the transcription of SI, LPH, and GLUT5. Analysis of the DNase I fingerprint of the rat LPH gene showed that the protected region retains the same sequence of *cis* elements (CE-LPH1) in the LPH gene (Goda, 2000).

At the gene level, it has also been discovered that AGP rs699 and IRS2 rs1805097 SNPs show a significant association with overweight individuals (BMI $P > 85$). The variants rs699, rs1805097, and rs17817449 were significantly associated with BMI and the variant of UCP3 rs1800849, with waist circumference. It is thus important in athletes to monitor these SNPs and not exceed in a high caloric meal (Muñoz et al., 2017).

Muscle energetic metabolism is a key process with genotypic diversity throughout athletes. In particular lactate production in anaerobic glycolysis is highly accumulated during high-intensity exercise and may influence the incidence of muscle injuries.

Lactate transport across the plasma membrane is mainly mediated by proton-linked monocarboxylate transporters (MTC1 and MTC4) that play a crucial role in intracellular pH homeostasis (Massidda et al., 2015). Genomic analyses revealed an association between lactate transporter defects in skeletal muscle and easy fatigue and muscle cramping upon exercise due to delayed removal of protons accumulated during anaerobic work and consequentially and higher risk for muscle injuries. A well-known polymorphism in the MCT1 gene (rs1049434 A/T) is associated with higher lactate levels in response to high-intensity training in subjects carrying the TT genotype because of an increased H+ concentration during exercise which lead to metabolic acidosis (Cupeiro et al., 2010; Fedotovskaya et al., 2014). This factor might compromise extreme performance in healthy individuals or athletes. Indeed, Fedotovskaya et al. (2014) have shown that the TT genotype and T allele are significantly under-represented in endurance athletes compared to controls. Despite controversial, in severe metabolic acidosis bicarbonate therapy seems to be beneficial (Sabatini and Kurtzman, 2009). In our context, we can hypothesize a utility of bicarbonate administration to individuals carrying the rs1049434 TT genotype of the MCT1 gene in order to reduce H+ concentration and improve endurance performance.

Recently, a work by Collins underlined the role of the AMPD1 rs17602729 gene polymorphism in exercise-induced myopathy and increased perceived pain posttraining. People carrying at least one T variant require longer rest periods between bouts of weight training. Adenosine monophosphate deaminase (AMPD) is a very important regulator of muscle energy metabolism during exercise playing a central role in the salvage of adenine nucleotides as well as the determination of energy charge. It has been shown that physical activity lowers skeletal muscle AMPD activity and part of the population who express the mutant AMPD1 T allele [2% of the Caucasian population are homozygous (TT genotype) and approximately 20% are heterozygous (CT genotype)] are vulnerable to muscular cramps, pain, and premature fatigue during exercises (Collins, 2017; Ginevičienė et al., 2014). A nutrigenomic approach to people carrying almost a T allele in AMPD1 gene might involve carbohydrate drinks for endurance athletes and creatine monohydrate supplementation for strength athletes in order to reduce muscle soreness and to accelerate muscle recovery.

18.2.2 Lipids

Genetic variability in lipid regulating genes has been studied in the last decades and the influence of these genetic polymorphisms on lipid plasma levels can suggest new dietary strategies to personalize a balanced diet regimen for athletes.

It is well known that the effect of dietary changes on plasma lipid concentrations differs significantly between individuals (Jacobs et al., 1983). Some individuals appear to be relatively insensitive (hyporesponders) to dietary intervention, while others

(hyperresponders) have significant improvement (Loktionov, 2003). In this regard, low-fat diets can cause reduced HDL (Katan et al., 1997) which can be particularly harmful to some subjects. For example, it has been shown that individuals with a predominance of small dense particles of LDL (subclass model B), a phenotype that is associated with an increased risk of coronary heart disease, benefit more from a low-fat diet (Jansen et al., 2002) compared to those with the subclass A variant (larger LDL).

Currently, there is considerable support for the idea that interindividual variability in response to dietary changes is determined by genetic factors, especially for different phenotypes for lipids and lipoproteins (Bertolini et al., 2004). Indirect evidence to support this hypothesis stems from the general observation that the phenotypic response to the diet is determined in part by the baseline value of the phenotype itself and that it is influenced by genetic factors (Williams et al., 1986). However, taking into account the complexity of lipid metabolism, the main problem is how to identify and clarify the many potential interactions between genes and diet.

Blood lipid response to a given dietary intervention could be dependent on the effect of both diet and gene variants. Brahe et al. investigated SNPs and diet mutual role in blood lipid profiles for 240 SNPs in 24 candidate genes, selected for their involvement in lipid metabolism pathways, and identified one significant interaction between LPIN1 rs4315495 and dietary protein that resulted in a decrease in TAG concentration for minor allele carriers on the high-protein weight maintenance diet. Adjusting for multiple testing, no other effects of SNPs or SNP-diet (protein content or GI) mutual contribution on blood lipid profile were detected after weight loss or after the 6-month ad libitum weight maintenance diet. People carrying this SNP should improve their protein intake in diet to prevent TAG increases especially for athletes with high caloric diet (Brahe et al., 2013) (Fig. 18.1).

An important and well-recognized factor in sports performance is the PPARα gene which encodes the peroxisome proliferator-activator receptor alpha, a central regulator of expression of other genes involved in fatty acid metabolism, which has been found positively associated with endurance athlete status. PPARα shows increased expression in tissues involved in fatty acids utilization such as liver, skeletal muscle, and cardiac muscle. Activation of PPARα promotes uptake, utilization, and catabolism of fatty acids by upregulation of genes involved in fatty acid transport, as well as in peroxisomal and mitochondrial fatty acid metabolism. Differences in genotype distribution of PPARα polymorphism between professional soccer players and sedentary volunteers have been investigated by Proia et al. In this study the authors found some variations in genotype distribution of the rs4253778 PPARα polymorphism between professional soccer players and sedentary volunteers. In particular, the G allele was significantly more frequent in soccer players compared with healthy controls, as was the GG genotype; No significant correlations were found between lipid profile and genotype background (Proia et al., 2014).

It is well-known that dietary PUFAs have effects on diverse biological processes such as insulin action, cardiovascular function, neural development, and immune function,

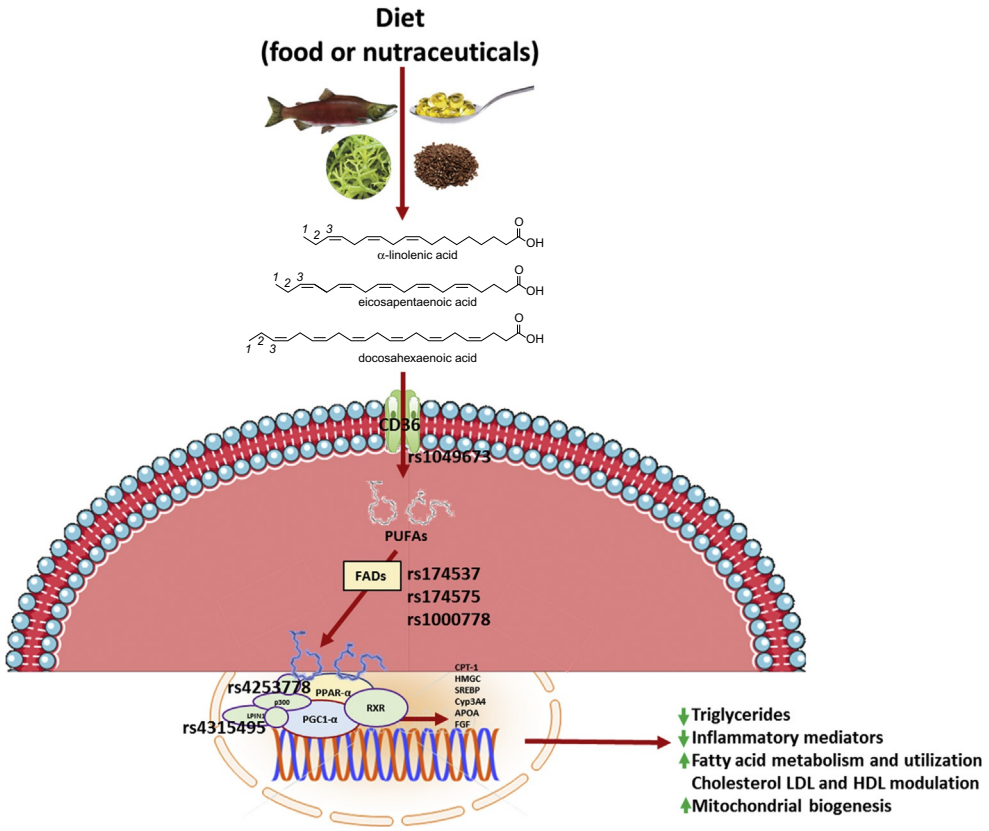

Fig. 18.1 Nutrigenomics of dietary PUFAs. Polymorphisms (SNPs) affecting PUFAs kinetics and dynamics.

some of them mediated via PPARα. Additionally, dietary PUFAs (in particular omega-3 fatty acids) activate, both directly and indirectly, other transcription factors such as liver X receptor, hepatocyte nuclear factor-4, and sterol regulatory element binding protein, which in turn mediate to some extent other biological processes affecting the expression of specific genes.

Health benefits of omega-3 fatty acids have been recognized for more than 40 years; however, there are gaps of knowledge in their mechanisms of action. This is due to the complexity of the action of omega-3 fatty acids, that have pleiotropic effects at the cellular level and act directly or indirectly through a wide range of metabolic pathways. Recently, nutrigenomics has contributed to a better understanding of the molecular mechanisms linked to the health benefits of omega-3 fatty acids. The use of nutrigenomics in this research field has been successfully applied in various human and animal models. For example, several detailed analyses of omega-3 PUFAs nutrigenomics have recently been

published using human blood mononuclear cells (Bouwens, 2009; Rudkowska et al., 2013), and on different tissues in animal atherosclerosis models (Gladine et al., 2012, 2014) that have contributed to a better understanding of the cardioprotective role of omega-3 PUFAs. All these studies show that the dietary intervention with PUFA omega-3 induces a substantial modulation of transcriptome, proteome, lipidome, and metabolome. Moreover, in all the experimental models used, the main pathways affected by the omega-3 PUFAs are related to inflammation, lipid metabolism or oxidative stress, while the main transcriptional regulators involved in these effects are NFκB and PPAR, both associated to crucial pathways in sports nutrition and athletic performance.

Other important data come from a study conducted by John P. Vanden Heuvel in which he concludes that diets rich in omega-3 fatty acids have long been associated with a reduced risk of CVD and prevention of some types of cancer. These effects could result from expression regulation of genes known to be "fatty acid receptors" (Skulas-Ray et al., 2010). It is estimated that genetic variation can explain a large portion of interindividual variability in o3-PUFA levels. FADS1 and FADS2 express the key enzymes involved in converting o3-PUFA into longer chain products. Several SNPs in these genes are associated with significant decreases in the percentage of o3-PUFA incorporated into serum lipids. Similarly, variations in genes encoding for other enzymes involved in the metabolism of fatty acids such as 5-LOX and polymorphic COX-2 can explain interindividual differences in levels and reactivity to o-PUFAs.

Many of the fatty acid receptors described above have genes with prevalent SNPs that are associated with the differential response to dietary intervention with o3-PUFA. For example, the carriers of the 162Val variant of PPARA and Ala12, the PPAR/G isoform generally respond to the supplementation of EPA and DHA with a greater reduction of serum triglycerides. Treatment with o3-PUFA is often associated with decreased circulating triglycerides and inflammatory mediators. However, the molecules responsible for producing beneficial responses vary within the population with polymorphic alleles in genes that encode lipoproteins such as APOE4 and cytokines like TNFα, among others. Indeed, several studies have shown an association between beneficial effects and polymorphisms of o3-PUFA in APOE, Gene FABP2, and TNF (Heuvel, 2012).

In terms of sports performance, it is widely recognized the importance of o-3 fatty acid in a personalized nutritional regime. Omega-3 fatty acids have to be taken at least after physical activity with a meal rich in lipid-like EVO oil or with proteins, better at night. The genetic variability should be addressed by varying the omega-3 daily intake depending on the genetic and lifestyles characteristics of the athlete.

18.2.3 Vitamins and minerals

An increasing number of studies in recent years have highlighted the importance of molecular nutrition as a potential determinant of health and disease (Jeukendrup and

Gleeson, 2018). In particular, the ability of minerals and vitamins to regulate the expression of genes and the production of proteins through the modulation of transcription and translation is now recognized. In sports nutrition, the daily amount of minerals and vitamins should be taken into consideration in order to provide the right dose of each micronutrient to each athlete in a personalized way. In particular, new nutrigenomic studies are now highlighting the important role of the correct daily intake of some minerals and vitamins to maximize sports performance and allow the body to recover properly after exercise (Jeukendrup and Gleeson, 2018).

18.2.3.1 Vitamin D

Vitamin D is a fat-soluble pro-hormone that can be consumed in the diet as cholecalciferol (vitamin D3) or ergocalciferol (vitamin D2) and activated in the skin in response to sunlight. Its nuclear receptor (VDR) modulates over 200 genes that transcribe proteins involved in maintaining bone health and calcium homeostasis, muscle function and mitochondrial antioxidant systems, autoimmune regulation, and synthesis of steroid hormones, among others.

About 200 polymorphisms in the vitamin D receptor gene (VDR) are known and others have been identified to be involved in vitamin D metabolism, therefore it is very easy to go into vitamin D deficiency for genetic reasons.

The expression of VDR decreases with age and the VDR genotype is associated with lean mass and strength in older men and women. Furthermore, there are strong relationships between vitamin D status and muscle function, especially in elderly patients. There is evidence that hypovitaminosis D is associated with a decline in muscle function. Vitamin D supplementation has beneficial effects on muscle strength, balance, and gait in various conditions although there are differences that can be explained with a dose of supplementation, a form of vitamin D and discrepancies in the threshold to define the deficiency/insufficiency (Beckett et al., 2014; Halfon et al., 2015; Potera, 2009; Puthucheary et al., 2011; bt Khaza'ai, 2018).

Vitamin D3 also plays a fundamental role in muscle with high-intensity exercise-induced damage and inflammation that are modulated by MAPK-NF-kB activation. High-intensity exercise activates p38 MAPK, which can stimulate nuclear translocation of p65 of NF-kB, as well as the gene expression of TNF-a and IL-6. Therefore, supplementation with vitamin D3 may be useful in rapid recovery or in protecting muscle damage from constant training (Choi et al., 2013).

Daily vitamin D intake is also essential for the modulation of insulin signaling and vitamin D can be used as a coadjuvant in the prevention of T2DM. However, vitamin D levels are responsible for the alteration of the pathophysiology of the DM2 gene due to the interaction of the mechanism of action VDR, DBP, 1-α-hydroxylase in skeletal and nonskeletal organs (Palomer et al., 2008).

It is thus important to monitor vitamin D3 plasma levels and to assess if some SNPs are presently known to be associated with the reduction of the amount of the active form of vitamin D3. It is suggested to perform a genomic analysis to assess vitamin D3 associated polymorphisms and quantify the daily dose of vitamin D3 needed.

18.2.3.2 Folate (as vitamer 5-methyltetrahydrofolate), methionine, choline and vitamin B

Folate (as vitamer 5-methyltetrahydrofolate), methionine, choline, and vitamin B12 are critical in carbon metabolism, which is the main source of methyl donors for cellular methylation reactions. These reactions include essential DNA synthesis and repair processes, DNA methylation, cell proliferation, and amino acid synthesis. Folate can be used in the de novo generation of methionine and, as such, the consumption of folates and other donors of methyl groups determine the availability of methyl groups for methylation reactions. Therefore, folates and other methyl group donors may indirectly influence miRNA profiles via alteration of DNA methylation status or may have an impact on miRNA expression levels through other methylation reactions. Altered functioning of catalytic enzymes in homocysteine (Hcy) metabolic pathways linked to gene mutations may lead to inhibition of certain pathways and elevation of plasma Hcy level (Ahmetov et al., 2016; Morton and Close, 2015).

Methylenetetrahydrofolate reductase (MTHFR), methionine synthase (MTR), methionines synthase reductase (MTRR), and betaine-homocysteine methyltransferase (BHMT) are enzymes in the Hcy remethylation pathway and interact with each other. Complex interactions between variations of corresponding genes, especially interactions between functional polymorphisms, can contribute to Hcy metabolic disorder and associated diseases. Sports nutrition is traditionally rich in protein and could alter the folate/Hcy pathways especially if not coupled with good vegetable intake. Thus, athletes should monitor this pathway measuring Hcy plasma level and through genetic analysis (rs1801133, rs1801394, rs1979277, rs1805087, rs18013940, rs492602, rs3733890, and rs6586282 SNPs associated to the folate/Hcy pathway thus introducing folic acid or directly the active form methylfolate and vitamin B6 and B12 with diet or supplements to prevent hyperhomocysteinemia (Suidasari et al., 2017).

Low folate consumption has been associated with an increased risk of muscle soreness and fatigue, muscle breakdown, and muscle strength. Vitamin B6 has also been found to be essential for the expression of genes useful for the repair and health of skeletal muscle (Beckett et al., 2014).

18.2.3.3 Minerals

Dietary trace element supplementation can result in an improvement in athletic performance. Athletes have a higher than normal requirement for minerals and an inadequate diet directly affects athletic performance.

Both iron deficiency and magnesium deficiency can result in a significant reduction in exercise performance. There is evidence that dietary magnesium intake may be suboptimal in some individuals, thus dietary supplementation of this element may be useful in some population groups. At the same time, iron supplements can improve athletic performance in individuals severely deficient in this element. If iron supplements are used, it is important that the level of supplementation is not excessive, as excess iron in the diet can result in an induced zinc deficiency (McDonald and Keen, 1988).

Interindividual variation in iron uptake and metabolism could be explained by polymorphisms in genes governing iron homeostasis. Several studies have examined the hemochromatosis (HFE), transferrin receptor-1 (TFR1), and TMPRSS6 genes in relation to iron storage and absorption. SNPs in these genes were strongly associated with lower serum iron concentration and other hematological variables (Wallace, 2016).

In marked contrast to iron and magnesium, there is little evidence for the idea that zinc deficiency influences exercise performance in humans. Despite this fact, zinc supplements have been widely advocated for the athlete, on the basis that intense exercise can result in changes in zinc metabolism. If zinc supplements are used, it is important that they are not excessive, as excess zinc in the diet can result in a secondary copper deficiency (McDonald and Keen, 1988).

Zinc, copper, and manganese are essential cofactors for the enzyme superoxide dismutase (SOD). SOD is a major antioxidant enzyme, which plays a vital role in the clearance of ROS. Among the isoforms of SOD, copper-zinc superoxide dismutase (SOD1, CuZn-SOD) with copper (Cu) and zinc (Zn) in its catalytic center is localized in the intracellular cytoplasmic compartments, and manganese superoxide dismutase (SOD2, Mn-SOD) plays an important role as a primary mitochondrial antioxidant enzyme. Numerous SOD polymorphisms have been detected, but only a few SNPs have been shown to have an impact in clinical practice. SOD1 +35A/C (rs2234694) which is located adjacent to the splice site (exon3/intron3 boundary), SOD2 Ala16Val (rs4880) which has been suggested to alter protein structure and function (C/T substitution in exon 2, codon position 2, amino acid position 16) and catalase −21A/T (rs7943316) which is located inside the promotor region just proximal to the start site, are the main SNPs. During physical activity ROS production increases and at the same time, antioxidant enzymes activity increases. Athletes carrying SNPs in the SOD enzymes might not be able to down-modulate excessive oxidative stress, which can lead to muscle damage in the long term. Another aspect is prolonged hyperglycemia due to genetic background or high caloric diet, which increases reactive oxygen species and modifies structure and function of lipids, proteins, and other molecules taking part in chronic vascular changes. Low activity of scavenger enzymes such as in people with SOD polymorphisms can accelerate this pathological condition leading to chronic diseases such as diabetes. In these athletes, supplementation with zinc, copper, and manganese might be interesting to

normalize SOD activity and prevent excessive oxidative stress and related-chronic conditions (Beckett et al., 2014; Flekac et al., 2008).

Nutrigenetics and nutrigenomics are comparatively new tools with which to study micronutrients. A critical evaluation of available data, incorporating omics technologies, strongly suggests that the intake or dietary supplementation with micronutrients should be optimized at an individual level.

18.2.3.4 Focus: *Caffeine ergogenicity and sports performance in athletes*

Caffeine (1,3,7-trimethylxanthine) is one of the most widely used performance-enhancing drugs. The performance-enhancing effects of caffeine have been known for over 100 years and are well replicated in both endurance-based activities and repeated high-intensity efforts (Guest et al., 2018).

The ergogenic effect of caffeine is related to several different mechanisms including a competitive adenosine receptor antagonist which reduce adenosine's downregulation of arousal and nervous activity and increase neurotransmitter release and muscle firing rates, an increase in adrenaline secretion and cellular ion release, and a decrease in pain perception. All of these mechanisms may improve caffeine ergogenic effects (Pickering and Kiely, 2018).

In terms of kinetics, plasma caffeine concentrations appear at about 15 min post-ingestion, peaking after about 60 min, with a 3- to 4-h half-life followed by an extensive liver metabolism by cytochrome P450 enzymes (primary CYP1A2), into paraxanthine, theophylline, and theobromine.

The general recommendation suggests the ingestion of 3–6 mg/kg of caffeine approximately 60 min prior to exercise despite recent research has shown high inter-individual variation in the ergogenic effects of caffeine with a wide variety of caffeine doses and timings.

The responses variations following caffeine ingestion are polygenic phenomena, mediated by multiple interacting genes and some genome-wide association studies have discovered SNPs associated with this behavior (Rahimi, 2018).

An SNP within CYP1A2 gene, rs762551, affects the speed of caffeine metabolization. Individuals with AA homozygotes ("fast metabolizers") tend to produce more of this enzyme and therefore metabolize caffeine more quickly. Conversely, C allele carriers ("slow metabolizers") tend to have slower caffeine clearance. The variable effects of this SNP are most well established in regard to health, with myocardial infarction and hypertension risk increased in slow metabolizers consuming moderate (3–4 cups) amounts of coffee, whilst fast metabolizers exhibit a protective effect of moderate coffee consumption. These studies prompted attention to how the CYP1A2 polymorphism might alter caffeine ergogenic effects in sports performance (Pickering and Kiely, 2018).

Womack et al. showed a significant effect of CYP1A2 genotype on the ergogenic effects of caffeine in trained male cyclists, with AA genotypes displaying a significantly

greater performance improvement than C allele carriers through two 40-km cycle time trials, following consumption of either 6 mg/kg of caffeine or placebo 60 min beforehand. These findings suggest that caffeine has a greater ergogenic effect for CYP1A2 AA genotypes than C allele carriers (Womack et al., 2012).

Recently, Guest et al. demonstrated that both 2 and 4 mg/kg caffeine improve 10-km cycling time in athletes carrying the AA genotype with no effect in those with the AC genotype and diminished performance at 4 mg/kg in those with the CC genotype concluding that CYP1A2 genotype might be examined when deciding whether athletes should use caffeine for enhancing sport performance and in which dose (Guest et al., 2018).

In conclusion, there is substantial variation in sports performance between individuals following caffeine ingestion. Understanding these differences, in part related to genetic variation between individuals, might lead to the development of personalized caffeine consumption guidelines for athletes.

18.3 Phytonutritional epigenomics: Role of phytonutrients in athletic performance

Epigenomics is the study of the complete set of epigenetic modifications on the genetic material of a cell, known as the epigenome. Epigenetic modifications are reversible modifications on a cell's DNA structure or histones that affect gene expression without altering the DNA sequence. DNA methylation, histone modifications, chromatin remodeling, noncoding RNA, are deeply interconnected layers all playing a role in epigenomic modifications that ultimately affect DNA expression (Romani et al., 2018). Epigenetic characteristics are specific to the individual and can represent key molecular patterns predisposing toward higher or lower physical performance capacities. By the same token epigenetic effects may also play a role in athletic potential (Ehlert et al., 2013).

Molecules with epigenetic effects can potentially be used to manipulate the epigenome and thus modulate DNA expression, by acting on one or more of the players of the "epigenetic orchestra" and of their mutual interactions, thus achieving genotype-phenotype interactions of therapeutic interest. In sports nutrition, an epigenomic approach could be applied to reduce muscle inflammation, to faster muscle recovering or to stimulate endogenous antioxidant systems. Given their multiple molecular effects, plant-derived molecules or whole phytocomplexes could be used to achieve a personalized therapeutic approach (Buriani and Fortinguerra, 2015; Buriani et al., 2017). Plant-derived molecules such as curcumin and resveratrol can modulate transcription factors (Nf-kB, NRF2, PGC1-a, FoXO3, AMPK, Sirt1) which lead to the transcription of key proteins involved in mitochondrial biogenesis, antioxidant systems, glucose and lipid homeostasis, and DNA repair which, in turn, ameliorate sport performance (Mead, 2007; Balogun et al., 2003; Lagouge et al., 2006).

Two important examples of the use of phytocomplexes or plant-derived molecules are provided by curcumin and resveratrol. Curcumin (diferuloylmethane), is a component of the spice turmeric (*Curcuma longa*) phytocomplex, which has been part of the traditional Asian medicine for centuries. In sports nutrition, turmeric and in particular curcumin may be potentially useful to prevent loss of muscle mass. Inhibition of NF-kB activity is of particular interest for the potential use of curcumin in the treatment of muscle wasting since NF-kB activation is a key step in the pathway leading to loss of muscle mass (Alamdari et al., 2009).

In a work by Delecroix et al. (2017), supplementation with curcumin and piperine each day between 48 h before and 48 h after exercise-induced muscle damage (EIMD) showed an effect on the recovery of some aspects of the muscle function. When the recovery period between competitions was short, a curcumin and piperine supplementation could be an effective recovery strategy to attenuate muscle damage. The recovery in sprint mean power output was moderately faster in the condition where the players consumed curcumin and piperine rather than placebo (Delecroix et al., 2017).

Another work assessed the effect of curcumin in EIMD and delayed onset muscle soreness (DOMS), which impact subsequent training sessions and activities of daily living (ADL) even in active individuals. In sedentary or diseased individuals, EIMD and DOMS may be even more pronounced and occur even in the absence of structured exercise. Curcumin supplementation resulted in a significantly smaller decrease in CK (−48%), TNF-α (−25%), and IL-8 (−21%) following EIMD compared to placebo supporting the use of oral curcumin supplementation to reduce the symptoms of EIMD (McFarlin et al., 2016).

Resveratrol (3,5,4′-trihydroxystilbene) is a natural phytoalexin which has been shown to improve oxidative stress levels in skeletal muscles of aged rats. As muscle disuse and reloading after disuse increases oxidative stress, resveratrol supplementation could improve muscle regeneration after disuse. Bennet et al. demonstrated the effect of resveratrol in improving muscle mass after disuse in aging (Bennett et al., 2013).

Another work by Sun et al. showed that exercise combined with resveratrol supplementation exhibit antiobesity functions in the long term due to enhanced mitochondrial biogenesis. This effect could be exploited by athletes aiming to reduce body fat and increase lean muscle mass. In order to obtain optimal resveratrol plasma concentrations, at least 200 mg of trans-resveratrol should be orally given at morning (Sun et al., 2018; Wang and Sang, 2018).

Another important aspect of resveratrol supplementation in sports performance is the effect on glucose control and insulin sensitivity. The latter is a new hot topic in bodybuilding, and there are some very valid reasons for that. In fact, one of the most important physiological tasks during a physical transformation like bodybuilding is making your body use insulin as much efficiently as possible, and resveratrol seems to be a promising molecule for this. A meta-analysis of 11 randomized controlled trials aimed to quantitatively evaluate the effects of resveratrol on glucose control and insulin sensitivity.

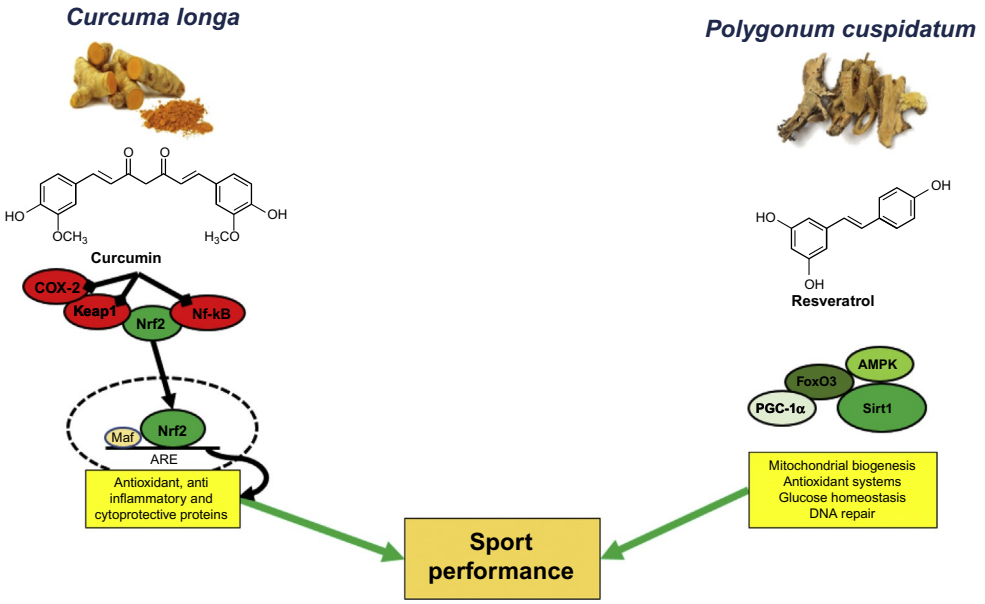

Fig. 18.2 Phytonutritional epigenomics of curcumin and resveratrol.

Resveratrol significantly improved glucose control and insulin sensitivity in persons with diabetes or prediabetes but did not affect glycemic measures in nondiabetic persons. These results suggest that resveratrol can be useful for those athletes who have high glycemic index and insulin resistance to ameliorate insulin actions in muscle absorption and growth (Liu et al., 2014; Baur et al., 2006) (Fig. 18.2).

18.4 Conclusion

In sports nutrition, the use of genetic information to personalize a nutritional regime can be applied to maximize athletic performances. However, for the majority of nutrients, there are still very little data or none at all, on the role of genetic polymorphisms on their ADME and the impact of these in athletic performance. In many cases, the application of nutritional genomics to sports performance is extrapolated from data of single polymorphisms analyzed for other conditions, or in specific diseases, so it will be necessary to confirm the data in athletes.

Further, individual network analysis comparing gene-nutrient associations, and genetic polymorphisms should be performed to understand in deep the physiological global status of an athlete in order to personalize a nutritional effective regimen. These applications of genomics in sports will gradually improve as the availability improves of progressively more informed and complete -omics databases as well as systems biology-oriented software.

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CHAPTER NINETEEN

Metabolomics and proteomics as tools to advance the understanding of exercise responses: The emerging role of gut microbiota in athlete health and performance

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19.1 Introduction

19.1.1 New trends in integrative nutrigenomics

A quarter of a century has passed since the introduction of nutrigenomics as a new approach to advance research on the interaction of nutrition and genes, which increased the global use of omics tools to study the interactions of nutrients and bioactive food compounds with the whole genome as well as the resulting changes in a wide array of proteins and metabolites (Müller and Kersten, 2003). The evolution and integration of nutrigenomic sciences have allowed more light to be shed on the mechanisms by which dietary components exert their effects and to identify new robust biomarkers of food intake and disease onset and progression (Mathers, 2017). Together with the increasing knowledge of genetic variations among relevant genes, these advances have paved the way for the application of personalized nutrition (and medicine) strategies on an extraordinarily detailed molecular level (Mathers, 2017). However, currently, it is well accepted that the individual contribution of these technologies cannot sufficiently capture the entire complexity of a human being that, from a biological point of view, should be considered a complex holobiont entity, that is, the host and its symbiotic microorganisms (Catania et al., 2017; Karczewski and Snyder, 2018). Gut microbiota (GM), which represent a large number of microorganisms present in the gut, have coevolved with humans and are a source of signaling molecules that are received by the host, including the fermentation products of nondigestible carbohydrates, such as

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short-chain fatty acids (SCFAs), and pro-inflammatory molecules, such as lipopolysaccharides (LPSs) (Janssen and Kersten, 2015; Brial et al., 2018). Recent findings have strongly suggested that GM play an important role in maintaining an optimal health status of the host and that the alteration of GM composition is associated with the appearance of diseases such as obesity, cardiovascular disease, and metabolic syndrome (Janssen and Kersten, 2015; Brial et al., 2018). Therefore, the study of the genetic material of the microbial communities present in the human gut using high-throughput sequencing and bioinformatics techniques, which together are known as metagenomics, has emerged as a key complementary omics approach for better characterization of health status (Morgan and Huttenhower, 2012). Furthermore, GM characterization could result in improved metabolic regulation and health through dietary treatments and food supplements that selectively enhance the expansion of friendly bacteria in the gut (Sáez-Lara et al., 2016).

As a comprehensive approach to analyze biological samples, the data generated from omics studies are enormous, complex, and noisy and are computationally difficult to display and challenging to comprehend biologically (Misra et al., 2018). High-powered computers, sophisticated software and statistical methods are essential to analyze the vast amount of data generated by every single study, not to mention the challenges of integrating information from hundreds or even thousands of studies (Kerksick et al., 2015). In fact, although the importance of the integration of omics data has been realized in a broad range of research areas, including food and nutrition science (Kato et al., 2011), a successful combination of more than two omics datasets is very rare (Misra et al., 2018). Considerable progress has been made in systems biology, which is an integrative approach to holistically combine the data obtained by a wide set of omics techniques, such as transcriptomics, metagenomics, RNA deep-sequencing, mass spectrometry (MS), nuclear magnetic resonance (NMR), protein-protein interactions, and proteomics (Mardinoglu et al., 2018), due to substantial advances in bioinformatics, machine-learning and biological network modeling. It is expected that this progress will enable the scientific community to improve the interpretation of statistics from each independent omics study, to identify unexpected biological relationships within their original biological context and to improve the time resolution of data, such as the resolution in time course studies that directionally inform on dynamic biological processes (Buescher and Driggers, 2016). Recently, the cost and processing time for sample analysis have significantly decreased, which has allowed for more effort to be spent on unraveling very large omics datasets resulting from omics postintegration. As a consequence, we are now more capable of uncovering unprecedented views of cellular systems at the exquisite resolution, resulting in transformative insights into biological processes and events. More importantly, we can now state that meeting the challenge of converting integrated omics-based data into biologically meaningful explanations is much closer (Misra et al., 2018).

19.1.2 Omics and integrated omics in the advancement of understanding athletic performance

Exercise and athletic practice induce the stimulation of many biological networks due to the complex response of the body to perturbations in homeostasis and mechanical demands (Hawley et al., 2014). As such, we should transition from targeted approaches to global unbiased systems approaches to fill the gaps in our understanding of the biological systems of exercise (Hoffman, 2017). As previously mentioned, to take advantage of recent technological gains and shorten the time needed to map the complexity of genetic and environmental contributions to exercise research, we need to advance all potential omics fields.

The link between genetics and the environment in individual human athletic responsiveness is addressed by genomics methods, including the identification of new single-nucleotide polymorphisms (SNPs) (Hoffman, 2017), and the study of how epigenomics reprogramming, which is central to skeletal muscle metabolic regulatory control, through decoding global DNA methylation patterns and posttranslational modifications (PTMs) on regulatory chromosomal proteins can also be impacted by both acute and chronic exercise (Howlett and McGee, 2016; Tanaka et al., 2016; Sarzynski et al., 2017). For example, in skeletal muscle samples, exercise produced hypomethylation in the promoter regions of key exercise-regulated metabolic genes (e.g., PGC-1a, PDK4, and PPAR-d), which was accompanied by the concomitant overexpression of these genes (Barrès et al., 2012). Metagenomics also represents a starting point to examine the study of physical exercise as an emerging contributor to GM composition with many implications in performance exercise and athlete wellness (Weir, 2017). Although diet-microbiota interactions have been studied extensively, exercise-microbiota studies are emerging as a field of research that will need the integration and understanding of metagenomic data.

Transcriptomic profiles help to decipher how a previous set of genomic and epigenomic environmental response mechanisms cumulatively affect the transcription networks of genes regulated by exercise, such as those related to the plasticity of human skeletal muscle (Hoffman, 2017), and to identify potential biomarkers of adaptation to training as well as health benefits derived from sport (Vechin et al., 2018). More recently in sports science, the role of small noncoding RNA molecules, such as a wide set of microRNAs (or miRNome), as important contributors to the posttranscriptional modulation that is regulated by exercise, represents an interesting area for future research in the epigenetic control of exercise response (Flowers et al., 2015). Furthermore, exercise-induced transcriptomic changes are intimately linked to downstream alterations in protein translation and posttranslational regulation that occur in the hours and days following exercise (Hoffman, 2017). In fact, new proteomic technologies will make available protein profiles that will narrow analyses to the single muscle fiber level (Murgia et al., 2015). These new profiles of accessible biopsies (e.g., muscle and adipose

tissue) (Leggate et al., 2012) and plasma should contribute to the unraveling of tissue-specific and systemic proteome dynamics that occur with exercise. In addition, global PTM MS-based approaches, including phosphoproteomics and acetylomics, have recently started to uncover the transducers and profiles of secretomes, that is, the factors secreted from cells and tissues via autocrine, paracrine, and/or endocrine systems, as an emerging subfield of proteomics to study exercise-regulated signals (Catoire et al., 2014; Hartwig et al., 2014). Downstream of the proteome, we find the metabolome that in exercise research is the profile of all metabolites that in turn describe the metabolic networks impacted by different intensity interval training programs (Peake et al., 2014; Kuehnbaum et al., 2015). Metabolomics enables us to characterize the exercise-induced adaptations that affect an abundance of small-molecule metabolites serving a wide range of biological functions, such as biomarkers of human cardiometabolic fitness in skeletal muscle (Huffman et al., 2014) and aerobic capacity in serum (Lustgarten et al., 2013). The quantification of the metabolomes of biological fluids, such as plasma, urine, saliva, and sweat, from subjects exposed to a range of exercise programs elicited a subfield of exercise metabolomics called "sportomics" (Bassini and Cameron, 2014).

The final goal of integrating omics fields into sport science is similar to the most important challenge in the life sciences: the ability to prescribe personalized nutritional and athletic performance programs to athletes. To reach this goal, we need to overcome significant hurdles, and we need cross-disciplinary efforts from exercise physiologists, geneticists, biochemists, clinical trial managers, computational biologists, and bioinformaticians. Furthermore, we should consider all surrounding variables before starting any intervention, including the following: (i) environmental conditions, such as altitude, temperature, or atmospheric pressure (Edwards and Thiele, 2013); (ii) the mode, frequency, duration, and intensity of exercise; (iii) the suitability to collect multiple cellular/tissue sample to decipher cross-talk mechanisms since different tissues have both distinctive and redundant exercise-regulated pathways (Hoffman, 2017); (iv) sex, age, and genetic variability; (v) the willingness of the subject to participate in voluntary exercise (Kelly et al., 2015); (vi) the health and nutritional and training status of the subject, considering hereditary and environmentally acquired exercise variables; and (vii) the microbiome composition as an important emerging biomarker of tissue exercise-environment interactions (Mach and Fuster-Botella, 2017).

Remarkably, integrating omics data will also provide a better understanding of the responses to a specific exercise program and the underlying mechanisms and will facilitate the identification of biomarkers of physical fitness, chronic stress, fatigue, overtraining, cardiovascular risk, and inflammation, which can allow for the personalized design of the most suitable exercise-based interventions. This, in turn, could elicit maximal health benefits for each individual and help us to predict the success of exercise-related strategies to counteract or ameliorate some disorders (Hawley et al., 2014). Indeed, the whole-body signature of endurance versus resistance exercise adaptations in health and disease

can be used to pinpoint biomarkers of training status and disease progression. By integrating omics profiles and whole-body physiological data together with both signatures of exercise adaptation, we could obtain biological explanations for real individual responses that include genomic variability in responsiveness to training or performing a specific type of exercise, thus optimizing the health benefits induced by a particular exercise regimen (Hoffman, 2017).

Therefore, the standardization of exercise protocols is strongly suggested to obtain sound, comparable, and reliable data on physiological responses. In addition, in order to harmonize the data available to allow for future integration by compiling and comparing interactions between omics profiles—(epi)genomic, transcriptomic, proteomics, and PTM and metabolomics—and each standardized exercise protocol and by connecting acute exercise modifications with downstream targets, we agree with other authors that suggest the establishment of a multicenter consortia to access large subject populations and streamline multiple omics-based approaches with international partners focusing on specific exercise interventions and research questions (Hoffman, 2017).

In this chapter, the authors present a review of the newest trends in the use of metabolomics, proteomics, and metagenomics to achieve the following: (1) to provide new insights into biomarkers of performance exercise and to describe the biological processes and mechanisms affected by physical exercise and/or by the use of supplement and nutritional interventions to improve athletic performance; (2) to discuss the relevance of GM in athletic performance with particular emphasis on the evidence describing the exercise-induced modulation of GM and the potential role of GM in the regulation of gut-brain axis in athletes; and (3) to review current strategies to improve athletes' exercise performance and health through dietary supplements that are able to favorably shape the GM.

19.2 Metabolomics, proteomics, and biomarkers of athletic performance

19.2.1 The use of metabolomics and proteomics to unravel biological mechanisms of physical performance and to identify the benefits of nutrition

Physical exercise is intrinsically linked to locomotion, which is powered by the hydrolysis of adenosine triphosphate (ATP) in skeletal muscle tissue. Metabolic pathways regulating ATP synthesis are rapidly activated during intense exercise via the breakdown of creatine phosphate and during the conversion of glucose units, derived from intramuscular glycogen to lactate (Egan et al., 2016). During more than several minutes of continuous exercise, the mobilization of extramuscular substrates, mainly from the liver (glucose) and adipocytes (free fatty acids), supports skeletal muscle metabolism. Thus, different levels of physical exercise generate metabolic imbalances that can be measured through metabolomics techniques to better understand the mechanisms and pathways related to

these metabolic responses (Egan et al., 2016; Heaney et al., 2017). Earlier techniques provided mechanistic insights into the phenotypic changes observed with exercise training and enabled the development of targeted training and nutritional strategies to maximize adaptations for health and performance (Heaney et al., 2017).

Currently, more advanced techniques, such as untargeted metabolomics, offer the potential to discover novel biomarkers that can provide important information to exercise scientists. These biomarkers may generate additional valuable information about the metabolic regulation of exercise adaptation, which should be useful in the future refinement of training programs for performance athletics and/or for obtaining optimal health benefits. Moreover, they also allow for further investigation of the interaction among nutritional interventions, metabolic perturbations and chronic adaptations in exercise training (Heaney et al., 2017; Suárez et al., 2017). Thus, simple blood tests that could provide predicted values of physical fitness would be of great interest for both medical and sporting communities, providing robust indicators for monitoring an athlete or a person that cannot perform exercise during an injury or is avoiding excessive training in busy competition periods.

In this context, some researchers have already applied metabolomics approaches in sports and training fields. One of these studies was carried out by Pohjanen et al. (2007), who used four hundred serum metabolites to predict the pre- or post-exercise state and showed increased glycerol and decreased asparagine were the most important discriminatory metabolites after an exercise session. Additionally, differences in serum amino acid profiles and metabolites related to energy production and oxidative stress were also observed between trained and untrained populations by Yan et al. (2008) Lower levels of serum phosphatidylcholine and increased free choline in a comparison of distinct low- and high-fitness groups has been reported by Bye et al. (2012).

Some studies have shown changes in lipid-related metabolites and oxidation, such as one conducted by Nieman et al. (2013b), which found significant elevations in serum metabolites related to lipid metabolism after an intensified training period in which endurance athletes underwent a repeated running protocol. Similarly, the same authors (Nieman et al., 2014a,b) also found an increase in plasma linoleic acid oxidation products, 13-HODE and 9-HODE, in response to an acute 75-km cycling program.

Other studies more focused on fatigue reported different clusters of plasma correlated metabolites between prefatigue and postfatigue time points in a maximal exercise cycling test, with the most interesting finding being that free fatty acids and tryptophan contributed to differences in the plasma metabolome at the fatigue stage (Manaf et al., 2018). In this context, Yan et al. investigated the effect of Korean ginseng on professional athletes since it is usually used for alleviating fatigue. They reported the antifatigue effect of the Korean ginseng treatment through the modulation of eleven endogenous metabolites involved in lipid metabolism, energy balance, and chemical signaling, which alleviated strength-endurance training effects such as increased creatine kinase (CK) and blood urea

nitrogen and decreased hemoglobin. Specifically, after 15 days of training, 9-hexadecenoic acid and ribose levels sharply increased, whereas 3-methyl-2-hydroxybutyrate, mannose, and myoinositol levels remarkably decreased in the control rowers; however, 3-methyl-2-hydroxybutyrate, ribose, mannose, and myoinositol levels remained unchanged in the ginseng-treated rowers. After 30 days of training, levels of 3-hydroxybutyrate, suberyl glycine, 9-hexadecenoic acid, and several unidentified compounds were significantly elevated, whereas glyoxylate levels were highly decreased in the control rowers; however, levels of 3-hydroxybutyrate, suberyl glycine, 9-hexadecenoic acid, glyoxylate, and the unidentified compounds remained stable in the ginseng-treated rowers. In short, the levels of some endogenous metabolites changed due to the training but these abnormalities recovered with Korean ginseng treatment (Yan et al., 2018).

The use of dietary supplements for athletic performance, such as *N*-acetylcysteine, is also under study. As an example, Paschalis et al. (2018) found that *N*-acetylcysteine supplementation increased exercise performance and reduced oxidative stress only in individuals with low levels of glutathione by using a targeted metabolic approach measuring F2-isoprostanes and protein carbonyls, which are two systemic oxidative stress biomarkers. Corn and Barstow (2011) also investigated the effects of oral *N*-acetylcysteine administration on fatigue, critical power, and curvature constant (W′) in exercising humans. The accumulation of reactive oxygen species (ROS) is associated with muscular fatigue, and *N*-acetylcysteine is an antioxidant that can extend the time to fatigue. However, their effect seems to be dependent on exercise intensity because *N*-acetylcysteine was successful at extending the time to fatigue at 80% but not at higher work rates (Corn and Barstow, 2011).

Nieman et al. (2015) evaluated the effect of a nutritional intervention strategy based on banana and pear ingestion on exercise performance and recovery after 75 km of cycling. They found that the best-performance times, 5.0% faster for banana and 3.3% faster for pear ingestion compared to that with only water consumption, were associated with reduced cortisol, IL-10, and total leukocyte levels and increased blood glucose, insulin, and ferric reducing/antioxidant power (FRAP) levels. Moreover, metabolomics studies showed that 107 plasma metabolites, most of which were related to lipid metabolism, showed an approximately twofold decrease with banana and pear consumption and that there was an increase in metabolites related to banana and pear, such as fructose, fruit constituents, and sulfated phenolic metabolites, that are related to the elevated FRAP. The authors concluded that banana and pear ingestion improved cycling performance and attenuated fatty acid utilization and oxidation. Similarly, the same authors also investigated the influence of pistachios on performance and exercise-induced inflammation, oxidative stress, immune dysfunction, and metabolite shifts in cyclists. However, they found 4.8% worse performance after pistachio supplementation. Metabolomics analysis revealed significant interaction effects for 19 metabolites, especially raffinose,

(12*Z*)-9,10-dihydroxyoctadec-12-enoate (9,10-DiHOME), and sucrose. Raffinose and sucrose are translocated to the bloodstream during exercise due to increased gut permeability, and plasma raffinose correlated significantly with 9,10-DiHOME and other oxidative stress metabolites (Nieman et al., 2014a,b).

In the context of aerobic exercise, which is known to provide benefits to human health and to reduce all-cause mortality, Felder et al. (2017) found a decrease in serum serine and glutamate metabolite levels in response to exercise, whereas levels of sarcosine and kynurenine increased. Phosphatidylcholines were also altered, specifically, the levels of four species increased and three decreased. All significantly altered lysophosphatidylcholines and plasmalogens levels increased, whereas only one short-chain acylcarnitine decreased. Daskalaki et al. (2015) also performed studies on the urinary metabolome after aerobic exercise and highlighted the purine pathway, tryptophan metabolism, carnitine metabolism, cortisol metabolism, androgen metabolism, amino acid oxidation, and GM metabolites as being the most affected by aerobic training.

In regards to elite-level athletes, Al-Khelaifi et al. (2018) compared the serum metabolic profiles of elite-level athletes from different sporting disciplines. They found that gamma-glutamyl amino acids were significantly reduced in both high-powered and high-endurance athletes compared to those of their moderate counterparts, indicating an activation of the glutathione cycle in the former group. Moreover, high-endurance athletes exhibited increases in sex hormone steroid levels involved in testosterone and progesterone synthesis and decrease in levels of diacylglycerols and eicosanoids relative to those of their moderate counterparts. Finally, they reported that high-powered athletes had increased levels of phospholipids and xanthine metabolites compared to moderate-power counterparts. Additionally, Wang et al. (2015) investigated the urinary metabolome of Chinese half-pipe snowboarders after different exercises to monitor physical training through the assessment of the health, performance, and fatigue of these athletes. They found that metabolic profiles were altered during different stages of exercise, with lactate, alanine, trimethylamine, malonate, taurine, and glycine levels decreasing and trimethylamine N-oxide (TMAO) and phenylalanine levels increasing with higher amounts and intensities of exercise. Howe et al. (2018) performed untargeted metabolomic profiling of athletes on an 80.5-km simulated treadmill ultramarathon, and they found that a large number of amino acids decreased and that fatty acid metabolism was affected, as measured by an increase in the formation of medium-chain unsaturated and partially oxidized fatty acids and carnitine conjugates. They hypothesized that prolonged exercise stimulated the proliferation of peroxisomes, which provide a readily utilizable form of energy through the formation of acetylcarnitine and other acylcarnitines for export to mitochondria in the muscles and may serve to regulate the levels of oxidized metabolites of long-chain fatty acids.

Finally, metabolomics was also applied to investigate military training by Karl et al. (2017) since this kind of training provides unique insights into the metabolic responses

to extreme physiological stress induced by multiple environmental stressors and the role of nutrition in mediating these responses. The authors performed untargeted metabolomics profiling of plasma samples and found that out of 737 identified metabolites, 478 changed during the extreme training, including increased levels of free fatty acids and acylcarnitines and decreased levels of mono- and diacylglycerols. Additionally, increases in tricarboxylic acid cycle intermediates and branched-chain amino acid metabolites were also observed. They reported that these findings are consistent with an increase in energy metabolism, lipolysis, fatty acid oxidation, ketogenesis, and branched-chain amino acid catabolism during military training. The magnitude of the energy deficit induced by undereating relative to high energy expenditure, rather than macronutrient intake, appeared to drive these changes, particularly within lipid metabolism pathways.

19.2.2 PTMs of key proteins of energy metabolism in response to physical exercise

Energy metabolism is the process of generating energy (ATP) from nutrients and comprises a series of interconnected pathways that can function in the presence or absence of oxygen. Aerobic metabolism converts one glucose molecule into 30–32 ATP molecules. Fermentation or anaerobic metabolism is less efficient than aerobic metabolism.

Exercise is intrinsically related to energy metabolism (Westerterp and Plasqui, 2004), and one way cells solve the increased energy demand during exercise is by ramping up the synthesis of mitochondria, the cells' power generators. Because mitochondria are the major regulators of cellular energy metabolism, providing the vast majority of ATP for cellular activity, mitochondrial dysfunction has been linked to the pathogenesis of some metabolic disorders, including obesity and type II diabetes mellitus (T2DM) (Boudina et al., 2005; Bugger et al., 2010). Therefore, it is important to identify the factors that control mitochondrial function and energy metabolism and that regulate the adaptive metabolic responses required by the increasing energy demands associated with exercise.

Several studies have analyzed the effect of an exercise period on the expression pattern and PTM of multiple protein classes and revealed that approximately 90% of the proteins identified were associated with energy metabolism, comprising enzymes involved in glucose catabolism, ATP synthesis and glutamate turnover (Ding et al., 2006).

The catalytic capacity of an enzyme can be changed by the noncovalent binding of allosteric effectors and/or by covalent PTMs, which involves an alteration of the original chemical composition of a protein after its translation. During the PTM process, biochemical groups, such as acetyl, phosphate, methyl, ubiquitin, and various lipid and carbohydrate residues, can be attached to or removed from specific amino acids in proteins. PTMs are one of the most important mechanisms for activating, changing or suppressing protein functions and represent an important way to diversify and regulate enzymatic activity (Walsh et al., 2005). It is a well-orchestrated process because

each PTM requires a specialized protein that catalyzes the particular modification, and some are reversible by the action of a specific protein.

Focusing on the most important PTM associated with the adaptive metabolic responses to acute exercise, a list of phosphorylated, acetylated, and ubiquitinated proteins will be described hereafter.

Phosphorylation—During muscle contraction, a number of stressors are induced (increased AMP/ATP ratio, ROS and lactate generation, Ca^{2+} flux, hypoxia, decreased energy availability) and collectively alter the posttranslational status of key cell signaling kinases. In the first global analysis of the phosphoproteome of human skeletal muscle in healthy subjects, 367 phosphorylation sites in 144 phosphoproteins were described. More than one-quarter were sarcomeric proteins from the contractile apparatus. Other phosphorylation sites were identified in some enzymes of glycogen metabolism and in some kinase and phosphatase subunits that regulate the phosphorylation of glycogen synthase and glycogen phosphorylase (Lundby et al., 2012). In mitochondria, other authors identified 155 distinct phosphorylation sites in 77 mitochondrial phosphoproteins that are mainly involved in oxidative phosphorylation (OXPHOS) (the most abundant), the TCA cycle, fatty transporters, β-oxidation, amino acid degradation, import machinery and transporters, calcium homeostasis, and apoptosis (Zhao et al., 2011). A recent global phosphoproteomic analysis of human muscle in response to a single bout of intense exercise (10 min cycling at 90% of $\dot{V}O_2$ max) revealed 1004 unique exercise-regulated phosphosites on 562 proteins, including substrates of known exercise-regulated kinases [such as AMP-dependent protein kinase (AMPK), protein kinase A (PKA), calcium/calmodulin-dependent protein kinase (CaMK), mitogen-activated protein kinase (MAPK), and mammalian target of rapamycin (mTOR)], although the majority of these targets had not been previously related with exercise signaling (Hoffman et al., 2015).

The acute PTMs of cellular signaling kinases, such as CaMKII, p38MAPK, and AMPK, acting alone or in combination with each other, can subsequently activate downstream transcription factors and coactivators that exert regulatory roles in the coordination of the expression of both nuclear and mitochondrial-encoded proteins, such as the peroxisome proliferator-activated γ receptor coactivator (PGC-1α) (Egan et al., 2010). PGC-1α, the so-called 'master regulator' of mitochondrial biogenesis, triggers pathways that promote mitochondrial synthesis and regulate both mitochondrial activity and energy metabolism (Liang and Ward, 2006). The phosphorylation of PGC-1α on different sites results in increased activity and subsequent translocation into both the nucleus (Little et al., 2010) and mitochondria (Safdar et al., 2011) during acute exercise, where it recruits and coregulates multiple transcription factors that control skeletal muscle gene expression, including nuclear respiratory factor 1 (NRF-1), nuclear respiratory factor 2 (NRF-2), estrogen-related receptor alpha (ERRα), and mitochondrial transcription factor A (TFAM) (Egan and Zierath, 2013; Hawley et al., 2014).

Additionally, acute exercise also induces phosphorylation of the p53 protein, a potent regulator of mitochondrial content, function, and biogenesis (Bartlett et al., 2014). The phosphorylation of p53 on serine 15, typically associated with increased stability and activity, occurred in parallel with the classical exercise-induced phosphorylation of both AMPK and p38MAPK, thus suggesting that these kinases may serve as upstream kinases modifying p53 activity.

Acetylation—A proteomics study in mouse liver mitochondria comparing sedentary, forced endurance exercise, and forced endurance plus 3-h recovery groups identified 277 acetylation sites on 133 mitochondrial proteins (Safdar et al., 2011). Deacetylases play an important role in energy metabolism. One of the well-established Sirtuin 1 (SIRT1) metabolic substrates is PGC-1α, which when it is deacetylated and activated, leads to a transcriptional switch from glycolytic to gluconeogenic genes in the liver and thus increases hepatic glucose production. In the liver and skeletal muscle, deacetylation of PGC-1α by SIRT1 induces the gene expression of fatty acid oxidation enzymes, and in a fasted liver, this deacetylation shifts the fuel usage from glucose to fatty acids (Gerhart-Hines et al., 2007).

SIRT1 also deacetylates other transcription factors, such as forkhead box protein O1 (FOXO1) and signal transducer and activator of transcription 3 (STAT3). Deacetylation of FOXO1, a crucial mediator of whole-body energy metabolism, activates target genes involved in gluconeogenesis in hepatocytes (Rui, 2014). Considering that PGC-1α serves as a coactivator for FOXO1, the deacetylation of both PGC-1α and FOXO1 by SIRT1 might have synergistic effects on their common gluconeogenic target genes. The transcription factor STAT3 acts as a negative regulator of gluconeogenesis, suppressing PGC-1α expression and thus inhibiting gluconeogenesis in the liver. The deacetylation of STAT3 by SIRT1 inhibits its phosphorylation and its translocation into the nucleus where it represses PGC-1α expression, and its suppression of gluconeogenesis is relieved (Nie et al., 2009).

Ubiquitination—Another important PTM that regulates mitochondrial energy metabolism is the ubiquitin-dependent degradation of mitochondrial proteins. The turnover of several OXPHOS proteins is dependent on the ubiquitin-proteasome system (UPS). Specifically, UPS-dependent degradation of succinate dehydrogenase subunit A (SDHA) promotes SDHA-dependent oxygen consumption and increases ATP, malate, and citrate levels (Lavie et al., 2018). A study performed in men exposed to divergent modes of exercise training and a single bout of exercise performed in the trained state demonstrated that prolonged traditional endurance and resistance training would both stimulate upregulation of basal levels of UPP molecular markers as a mechanism of muscle remodeling to maintain an optimal size of the type I fibers. These results suggest that adaptations due to endurance exercise training are more reliant on protein UPP degradation processes than adaptations due to resistance exercise training.

19.3 GM in athletic performance

19.3.1 Associations between exercise and alterations in GM and implications for the immune system

GM has been shown to exert multiple functions that ultimately influence host health status. The composition and function of GM can be altered by several external factors, such as diet, antibiotic exposure, or stress. Thus, diet is one of the main factors that modulate the composition of GM (Sonnenburg and Bäckhed, 2016), which is illustrated by the fact that adherence to extremely different diets is associated with the profound changes in GM among individuals from around the world (De Filippo et al., 2010). Recently, physical exercise has emerged as another potential driver of GM composition. The scientific data supporting this fact come mainly from preclinical studies that indicate that exercise promotes a higher microbiota diversity, an indicator of a healthier status, and favors the presence of beneficial bacteria in the gut (Cerdá et al., 2016; Monda et al., 2017). Some human studies have also observed similar results. A study in healthy active adults found a positive correlation between cardiorespiratory fitness and GM diversity, independent of their diet (Estaki et al., 2016). Moreover, the microbiome profile of fit individuals was related to a higher production of SCFA butyrate and an increased proportion of butyrate-producing GM taxa. Other studies have revealed an increased diversity in the GM of professional athletes when compared to that of sedentary adults (Clarke et al., 2014; Barton et al., 2018), although differences in dietary habits could also explain these differences. In contrast, an 8-week combined aerobic and resistance training in sedentary healthy adults did not induce a substantial modulation of GM composition and diversity, with only a slight increase in bacterial diversity. Hence, a recent study performed in young soldiers subjected to multiple-stressor military training characterized by extreme and prolonged exercise (4-day cross-country ski-march) and suboptimal energy intake led to marked alterations in GM composition and metabolism in parallel with increased intestinal permeability (Karl et al., 2018). However, another recent study performed on sedentary adults showed that an 8-week training regimen with moderate intensity and duration (three times per week) did not alter the GM composition, suggesting that the reported improvements in microbiota diversity and composition in elite athletes may represent late and cumulative responses to habitual exercise (Cronin et al., 2018). The effects of exercise on GM could be subjected to the health status of the individual because a 6-week endurance training program has been found to increase intestinal SCFA levels along with changes in SCFA-producing bacteria in lean but not obese subjects (Allen et al., 2018). Interestingly, these changes disappeared after the cessation of this exercise intervention.

Although the effects of exercise on GM composition have been clearly described, no well-defined microbial pattern associated with these effects has been established thus far. Bacterial genera or species reported to be influenced by exercise differ widely among

studies, as well as between animals and humans, probably due to substantial differences in study designs. Thus, further research is needed to firmly establish exercise-altered microbiome patterns.

The possible mechanisms that have been proposed to explain the modulation of GM by physical exercise include the following: modified bile acids; modified SCFA intestinal profile; reduced gut transit time and altered gut pH; increased secretion of intestinal immunoglobulin A (IgA); reduced stimulation of toll-like receptors (TLR) by bacteria-derived lipopolysaccharide; altered hypothalamic-pituitary-adrenal (HPA) axis through the bidirectional interaction of the GM and the brain; and exercise-induced weight loss (Cerdá et al., 2016; Estaki et al., 2016).

The GM could also be implicated in the immune-stimulating effects of physical exercise. The microbiota play an important role in the regulation of the immune system by the direct or indirect stimulation of dendritic cells, T regulatory cells and neutrophils, B cell maturation, and modulation of the expression of TLR (Bermon et al., 2015). The SCFAs produced by gut bacterial fermentation are also implicated in the regulation of the host immune and inflammatory responses (Bermon et al., 2015). Considering this connection between GM and the immune system, it is not surprising that an imbalance in the microbial equilibrium (termed dysbiosis) could be linked with immune alterations and the development of immune diseases, while increased microbiota diversity has been linked with improved immunological responses.

The previously exposed effects of exercise on the gut microbial ecosystem, for example, increased SCFA production or bacterial diversity, could explain the beneficial effects of physical exercise on immunity. Moreover, physical exercise may enhance the sensitivity of TLRs, which are modulated by microbiota through the microbe-associated molecular pattern (MAMP) and play a critical role in innate immunity (Bermon et al., 2015; Deckx et al., 2016). Altogether, this suggests possible cross talk between exercise, GM, and immunity, although it has not yet been established.

However, while moderate and regular exercise may enhance immune function, prolonged high-intensity exercise training has been shown to negatively affect the immune system, increasing the risk of infections and allergic diseases (Silva and Moreira, 2015). In this sense, excessive training has been related to gut dysbiosis accompanied by increased gastrointestinal (GI) permeability and decreased mucous thickness (Mach and Fuster-Botella, 2017). In this situation, endotoxins such as LPS are able to cross the epithelial barrier into the bloodstream, leading to endotoxemia and provoking a disruption of the homeostasis between the immune system and GM, which results in the induction of the secretion of pro-inflammatory cytokines.

19.3.2 Exercise-induced stress behavior and the gut-microbiota-brain axis: Mood and fatigue

It has been estimated that between 20% and 60% of athletes (especially those participating in endurance sports) suffer from stress caused by excessive training and inadequate

recovery (Purvis et al., 2010). Strenuous exercise is accompanied both by physical and psychological stress, both of which exert similar effects. Common symptoms associated with stress are inflammation, immunosuppression, fatigue, insomnia, weak performance, low appetite, weight loss, and mood disturbances such as irritability, anxiety, loss of motivation, depression, or poor concentration (Clark and Mach, 2016). Stress during exercise can stimulate several systems, such as the sympathoadrenomedullary (SAM) and the HPA axes, which results in the release of catecholamines and glucocorticoids (Clark and Mach, 2016). The HPA axis is considered the core stress efferent axis that coordinates the adaptive responses of an organism to stressors. Stress also activates the autonomic nervous system (ANS), which increases the neuronal release of norepinephrine (NE) and other transmitters in peripheral tissues such as the GI tract, which is innerved by the central nervous system (CNS) and the enteric nervous systems (ENS) within the gut wall. The reciprocal interaction between the gut and brain has long been recognized. This bidirectional gut-brain communication occurs along a network of pathways collectively known as the gut-brain axis. It encompasses the CNS, HPA, ANS, and ENS, as well as the neuroendocrine and neuroimmune systems. Accumulating evidence points toward the critical influence of the GM in the regulation of this axis (Cryan and Dinan, 2012; Carabotti et al., 2015). Because of this, the "gut-brain-microbiome axis" nomenclature has been suggested to be more inclusive and appropriate.

Stress and the associated activation of the HPA axis have long been shown to alter the composition of the gut microbiome, and as was previously mentioned, some studies have also shown that exercise-induced stress also alters the GM composition and intestinal permeability (Karl et al., 2018). Exercise intensity and duration are the two main factors involved in the regulation of exercise-induced HPA axis activation, which might partly explain the discrepancy in these results. Therefore, when the intensity of the physical exercise is above 60% of VO_2 max or the duration of exercise exceeds 90 min at low intensity (below 40% VO_2 max), there is an activation of the HPA axis, and hormones are released (Duclos and Tabarin, 2016). Similarly, in mice, it has been shown that only very intense running exercises above the lactate threshold induce significant HPA axis activation (Soya et al., 2007).

Because communication within the gut-brain-microbiome axis functions bidirectionally, microbiota might have an important role in modulating the stress response. In fact, the potential influence of the microbiome on CNS is currently a growing area of research, and recent studies have shown that microbiota are able to modulate stress-related behavior (Rea et al., 2016; Foster et al., 2017), although the specific role of microbiota in the adaptation to exercise-induced stress has not been demonstrated. The first study providing evidence of the role of GM in the development of the HPA stress response was conducted in 2004 (Sudo et al., 2004). This landmark study showed that adult germ-free (GF) mice displayed an exaggerated HPA stress response when subjected to mild restraint stress compared to the response of specific pathogen-free (SPF) controls.

Importantly, this enhanced stress response was partly age-dependent reversed by recolonization with control mice feces and totally reversed by GM colonization with *Bifidobacterium infantis*. These findings stimulated further research to elucidate the role of the intestinal microbiome in governing the stress response in GF animals and other models manipulating the GM through bacterial infections, prebiotics, and probiotics supplementation, and/or antibiotic treatment. In addition, confirming the GM influence on stress responsiveness, some of these studies showed that it also has an impact on stress-related emotional behavior, particularly anxiety and depression, and their respective underlying pathologies (Rea et al., 2016; Foster et al., 2017). These results highlight the bidirectional nature of the HPA axis.

This GM modulation of the gut-brain axis may occur through multiple direct and indirect mechanisms (Cryan and Dinan, 2012; Carabotti et al., 2015; Rea et al., 2016), including modulation of the intestinal barrier; modulation of afferent sensory neurons of the vagus nerve; alterations in the circulating levels of cytokines released from mucosal immune cells; modulation of tryptophan metabolism, a precursor to serotonin, which is a key neurotransmitter within the brain-gut axis; release of gut hormones such as serotonin from enteroendocrine cells (EEC); and the production of microbial-derived metabolites (SCFA, bile acids) and neurometabolites (e.g., GABA, noradrenaline, serotonin, dopamine). In particular, the modulation of tryptophan and serotonin availability may play a critical role in exercise-induced stress-related fatigue and mood disturbances, including depression and insomnia in athletes.

Fatigue is a complex phenomenon influenced by the interaction between peripheral and central factors. Peripheral fatigue refers to the muscle itself independent of the CNS, while central fatigue involves the failure of the CNS to adequately drive the muscle. The original central fatigue hypothesis suggested that increased brain serotonergic activity during prolonged exercise in several brain regions might cause sleep, lethargy, and drowsiness, thus increasing the mental effort necessary to maintain athletic performance and ultimately leading to fatigue and limiting performance. Although serotonin is usually associated with the brain, only 5% of the body's serotonin is produced in the neurons of the CNS (O'Mahony et al., 2015; Agus et al., 2018). The vast majority of its production (95%) occurs peripherally in the gut, where it is synthesized mainly in enterochromaffin (EC) cells (∼90%) and a major subset of EECs and to a lesser extent, in neurons of the ENS (∼5%). Synthesis of neuronal serotonin, both in the CNS and ENS, is regulated by the rate-limiting enzyme tryptophan hydroxylase 2 (TPH2), while EC cell-derived serotonin is produced by TPH1. Interestingly, several studies in GF mice have reported that GM are able to directly modulate serotonin production in the gut. Hence, GM can increase serotonin synthesis and release in EC cells by upregulating TPH1 expression (Yano et al., 2015). Although peripheral serotonin does not cross the blood-brain barrier, the GM could also indirectly affect brain serotonin by modulating tryptophan metabolism.

Tryptophan is the sole precursor of peripheral and central serotonin, but only 1%–2% of tryptophan is converted to serotonin via the serotonin pathway. The major catabolic route of tryptophan (>90%) is in the kynurenine pathway (KP), resulting in nicotinamide and NAD^+ (O'Mahony et al., 2015; Agus et al., 2018). Therefore, KP has a major influence on the availability of tryptophan for serotonin production. Tryptophan metabolism through this pathway is mainly mediated by indoleamine 2,3-dioxygensase 1 (IDO1), which is expressed in all tissues, and by tryptophan 2,3-dioxygenase (TDO), which is mainly expressed in the liver but also in the brain. Importantly, a critical role of the GM in influencing the activity of these enzymes has been demonstrated in preclinical models (O'Mahony et al., 2015; Agus et al., 2018; Gao et al., 2018). For example, in GF animals, KP metabolism (measured by the kynurenine/tryptophan ratio) was decreased and the concentration of hippocampal serotonin was significantly increased compared to those of conventional animals due to a microbiota deficiency, whereas recolonization of the GM increased KP metabolism (Clarke et al., 2013). On the other hand, TDO is activated by glucocorticoids, and IDO1 is stimulated by oxidative stress and inflammatory stimuli. IDO1 activation has been shown to alter gut microbial composition (Gao et al., 2018). Therefore, exercise-induced stress resulting in the activation of the HPA axis may activate the KP and potentially affect the composition of the microbiome. In fact, a recent study showed that the activation of the KP resulted in enhanced tryptophan catabolism and kynurenine production following exhaustive aerobic exercise (Strasser et al., 2016). Finally, GM can also directly regulate tryptophan availability, representing a third major pathway in exercise-induced fatigue signaling. Only 4%–6% of tryptophan is directly used by GM and converted to tryptamine or indole pyruvic acid (Agus et al., 2018). In addition to degrading, tryptophan, bacteria can also synthesize it, unlike humans (O'Mahony et al., 2015). The balance between these direct and indirect mechanisms of bacterial modulation of tryptophan metabolism and serotonin production will determine tryptophan availability for the host and eventually serotonin production.

19.4 Diet modulation of GM profiles to improve athletic performance: Potential ergogenic aids

The dietary regimen of athletes, which is generally based on a high consumption of simple carbohydrates, high to moderate consumption of animal protein, and low consumption of fat and fiber (American Dietetic Association et al., 2009), is likely the main explanation for their high GM diversity (Bermon et al., 2015; Clark and Mach, 2016). Although avoiding fiber can reduce GI discomfort by facilitating rapid gastric emptying, the very low intake of these plant polysaccharides in athletes can also negatively affect the diversity and functionality of the GM (Clark and Mach, 2016). This, in turn, can result in the decreased production of SCFAs, hormones, and neurotransmitters that are involved in the regulation of the HPA axis, which could lead to the impairment of athletic

performance (Clark and Mach, 2016). Furthermore, a high intake of proteins of animal origin can also exert negative outcomes on gut health because it has been shown by in vitro and animal models that some of the by-products of amino acid fermentation, such as amines and volatile sulfur compounds, can increase intestinal permeability and inflammation (Clark and Mach, 2016; Mach and Fuster-Botella, 2017). Remarkably, in a recent randomized placebo-controlled trial (RCT), Moreno-Pérez and collaborators showed that the intake of a protein supplement based on whey isolate and beef hydrolysate for 10 weeks diminished the abundance of the beneficial bacterial taxa *Roseburia*, *Blautia*, and *Bifidobacterium longum* in endurance runners (Moreno-Perez et al., 2018). Altogether, these findings suggest that decreased consumption of animal protein during resting days and training is recommended to ensure optimal GM and intestinal health in athletes (Clark and Mach, 2016). Thus, considering all these concerns, the manipulation of the GM toward an improved profile is emerging as a potential tool to improve athletic performance and health. Next, a review of the existing literature focused on dietary supplements that can act as direct or indirect ergogenic aids partly through modulation of GM and its derived metabolites are presented.

In recent years, probiotics, which are live microorganisms that confer health benefits to the host when they are administered in appropriate amounts, have appeared as a promising nutritional strategy to improve athletic performance (Bermon et al., 2015; Clark and Mach, 2016; Mach and Fuster-Botella, 2017; Colbey et al., 2018; Pane et al., 2018). In a 4-week study conducted with male mice, Hsu et al. recently demonstrated that daily supplementation of different doses of a kefir containing five bacterial strains of the genera *Lactobacillus* and *Streptococcus* increased exhaustive swimming time, forelimb grip strength, muscle mass accretion, and hepatic and muscular glycogen content and decreased the blood levels of the fatigue biomarkers lactate, ammonia, urea nitrogen, and CK (Hsu et al., 2018). Interestingly, these changes were accompanied by a decreased Firmicutes/Bacteroidetes ratio in the cecum samples of mice supplemented with the two higher kefir doses (Hsu et al., 2018). Since Bacteroidetes are associated with enhanced production of SCFAs through the catabolism of branched-chain amino acids (Boulangé et al., 2016) and lactic acid bacteria can use lactate to produce butyrate (Duncan et al., 2004), the authors hypothesized that the beneficial effects of kefir on exercise performance and fatigue could be partly driven by favorable changes in the GM, which would promote effective removal of blood lactate and increased nutrient availability through its conversion to butyrate. In another study performed in mice, Chen et al. reported that daily supplementation with *Lactobacillus plantarum* TWK10 (LP10) for 6 weeks increased grip strength, endurance swimming time, muscle mass, and decreased the blood levels of lactate, ammonia, and CK (Chen et al., 2016). Remarkably, the same group obtained positive results in an RCT carried out with sixteen 20- to 40-year-old amateur athletes who underwent exhaustive treadmill exercise, reporting that supplementation with LP10 for 6 weeks increased the endurance

performance and circulating blood glucose levels of subjects in a maximal treadmill running test compared with those of the subjects that received the placebo (Huang et al., 2018). Some of the potential mechanisms that could be responsible for the beneficial effects induced by LP10 are the bacterial production of butyrate from the lactic acid generated by *Lactobacillus*, which, in turn, would make possible the formation of ATP via the so-called classical pathway (Duncan et al., 2004); the antiinflammatory effects of LP10, leading to an improvement in skeletal muscle atrophy markers; and the promotion of glucose utilization by increasing the number of gastrocnemius type I muscle fibers (Chen et al., 2016; Huang et al., 2018). Nevertheless, further studies are needed to elucidate whether the beneficial effects of this probiotic are partly mediated by changes in the GM and microbial-derived metabolites. In a crossover RCT conducted with ten trained male runners, daily supplementation with a probiotic supplement containing *Lactobacillus*, *Bifidobacterium*, and *Streptococcus* strains for 4 weeks increased the run time to fatigue in hot conditions (35°C and 40% humidity) (Shing et al., 2014). The authors suggested that the mechanisms responsible for this enhancement of exercise performance could be related to the beneficial effects of probiotics on intestinal immune function, architecture, and integrity because they found slight, but not significant, reductions in GI permeability and endotoxemia (measured as serum levels of LPS) at rest and postexercise and found a trend toward increased postexercise IL-10 blood levels following probiotic supplementation (Shing et al., 2014). The findings of Shing et al. (2014) contradict those found by Cox and collaborators, who in a parallel RCT conducted with twenty highly trained distance runners, did not find differences in training duration or intensity between subjects receiving a probiotic capsule containing *Lactobacillus fermentum* for 28 days and those that were supplemented with a placebo (Cox et al., 2010). The discrepancies between these two RCT studies may be due to the bacterial strains used, the daily dose (45 times lower in Cox et al.), the length of the treatment and the differences in the exercise test used to determine athletic performance.

Despite the undoubted interest of the aforementioned findings, the majority of the available scientific data do not support the use of probiotics as ergogenic aids that directly enhance exercise performance. In contrast, most articles indicate that probiotics can act as indirect ergogenic aids due to their immunomodulatory and antiinflammatory properties and their ability to improve the integrity of mucosal barriers. Similarly, probiotics have been recently used to ameliorate and prevent upper respiratory tract infections (URTIs) and persistent common cold and flu-like symptoms in athletic cohorts, although the results are not completely consistent (Mach and Fuster-Botella, 2017; Colbey et al., 2018). Thus, in a parallel RCT, daily supplementation with the *Lactobacillus casei* strain Shirota by 42 endurance male and female athletes over a 16-week winter period of training and competition reduced the incidence and prevalence of URTIs, and these effects were accompanied by higher levels of salivary IgA (Gleeson et al., 2011). Positive

outcomes concerning the incidence and number of episodes of URTIs were also observed in elite male distance runners (Cox et al., 2010), physically active males and females (West et al., 2014) and female endurance swimmers (Salarkia et al., 2013) who took probiotics-based supplements. Interestingly, in this last study, probiotic consumption was associated with a significant increase in VO_2 max, probably due to the decrease in URTIs, which would support the use of this dietary supplement containing *Lactobacillus*, *Bifidobacterium,* and *Streptococcus* strains as an indirect ergogenic aid (Salarkia et al., 2013). In contrast, other RCTs did not reveal the beneficial effects of probiotic supplementation on the incidence of URTIs (Kekkonen et al., 2007; Gleeson et al., 2012). However, one RCT conducted with elite cyclists reported that supplementation with *L. fermentum* was effective in ameliorating URTI symptoms in males but was detrimental in females, which could tentatively explain, at least in part, the gender-dependent effects of the supplement on modulation of gut microflora, quantified by increased fecal content of *Lactobacillus* in men but not in women (West et al., 2011). As a result of the documented beneficial effects of probiotics against diarrhea in children (Bertelsen et al., 2016) and the various studies that have shown that these live microorganisms are able to improve the barrier function of the intestinal mucosa (Stoidis et al., 2011), probiotics are also in the spotlight as a nutritional therapy to prevent or ameliorate the gastrointestinal disorders (GD) that often occur during heavy training and competitions (Mach and Fuster-Botella, 2017). In a crossover RCT conducted with 30 elite rugby players, the daily supplementation of a probiotic capsule containing *L. gasseri*, *B. bifidum*, and *B. longum* significantly for 4 weeks decreased the incidence of GD and URTI, which was demonstrated by the fact that 14 out of 30 participants never experienced a single GI or URTI episode during probiotic treatment compared to 6 out of 30 in the placebo group, and the number of days of illness was slightly lower when the athletes took the probiotic (Haywood et al., 2014). Similarly, Kekkonen et al. (2007) reported that in marathon runners that were supplemented with *L. rhamnosus* GG during a training period of 3 months, the duration of GI episodes was significantly decreased during the 2 weeks after the marathon compared with that of the athletes that received the placebo. Interestingly, in a parallel RCT conducted with runners, cyclists, and triathletes, Lamprecht et al. (2012) found that daily supplementation with a probiotic supplement containing six probiotic strains of the genera *Bifidobacterium*, *Enterococcus*, *Lactobacillus*, and *Lactococcus* for 14 weeks significantly decreased the fecal levels of zonulin, a marker of gut permeability, and the circulating levels of the tumor necrosis factor α (TNFα), suggesting that these live microorganisms improved the integrity of the intestinal barrier. These findings could account for the beneficial effects of probiotics on decreasing endotoxemia and the associated cytokine production that can occur in athletes under sustained exercise stress. On the other hand, different animal and human studies suggest that probiotics can also decrease stress- and mood-related disturbances, such as depression and anxiety, although well-designed RCTs are needed to elucidate whether

the bioactive ingredients are able to exert beneficial psychological effects in athletes and to shed more light on how probiotics can modulate the gut-brain-microbiome axis (Clark and Mach, 2016).

Considering all the data, supplementation with probiotics could be a useful strategy to improve athletic performance and ameliorate the behavioral, respiratory and GD that can affect athletes. Nevertheless, scientific evidence of their effectiveness is still scarce, and the results are somewhat controversial. Differences in the bacterial strains, study design, sample size, length of treatment, daily dose, exercise test, type of sport and athlete training, and dietary background could account for the discrepancies in the effectiveness of probiotic-based supplements observed among RCTs. Therefore, new well-designed RCTs carried out with larger cohorts will be crucial to confirm the usefulness of this nutritional therapy. Furthermore, analyses of GM composition and microbial-derived metabolites are needed to unravel the mechanisms by which probiotics can exert healthy effects in athletes. Obtaining new robust scientific evidence would pave the way for the incorporation of probiotics into diets aimed at improving athletic performance by enhancing healthy microbiota communities and metabolites and for the establishment of clinical and practical guidelines.

Due to their ability to favorably shape the GM, polyphenols, which are biomolecules present in fruits and vegetables with antiinflammatory and antioxidant properties (Tresserra-Rimbau et al., 2018), and prebiotics, nondigestible food ingredients that confer health benefits to the host when fermented by selectively enhancing the growth and/or activity of beneficial bacteria in the gut (Bertelsen et al., 2016), are other potential candidates to provide beneficial effects for exercise performance (Mach and Fuster-Botella, 2017). Concerning prebiotics, a recent 16-day study conducted with mice induced to exhaustive exercise stress demonstrated that daily supplementation with neoagarotetraose (NAT), a neoagaro-oligosaccharide of marine origin, significantly reduced markers of fatigue induced by intense exercise, as measured by significant increases in blood glucose and hepatic glycogen and decreases in lactate and the oxidative stress marker malondialdehyde (Zhang et al., 2017). Interestingly, the administration of NAT to these mice also counteracted the alteration of intestine epithelial integrity and the decrease in fecal content levels of SCFAs acetate, propionate, and butyrate observed in the mice submitted to a forced exercise wheel-track treadmill relative to that observed for mice that received a placebo (Zhang et al., 2017). These NAT-induced changes were also accompanied by an improvement of the GM profile and a decrease in the relative abundance of different potential pathogens present in mice that underwent intense exercise (Zhang et al., 2017). Altogether, these results suggest that NAD could be potential ergogenic acting through the modulation of the GM and microbial-derived metabolites. In humans, the limited scientific evidence available suggests a beneficial role of prebiotics as indirect ergogenic aids, improving URTIs and immunity (Bergendiova et al., 2011; McFarlin et al., 2013). In healthy active subjects,

supplementation with β-glucan for 28 days after completing a marathon was associated with a 37% drop in the number of cold/flu symptom days, whereas the same treatment for 10 days significantly increased the salivary IgA 2 h after 50 min of strenuous cycling in a hot and humid environment (McFarlin et al., 2013). In another RCT carried out with 50 regularly training subjects that received a β-glucan for 3 months, a decrease in the incidence of URTI symptoms, an increase in the blood levels of natural killer cells and improvement in the phagocytosis process were observed (Bergendiova et al., 2011). In contrast, Nieman et al. did not find beneficial effects of β-glucan on URTIs and immunity in trained male cyclists that received this supplement 2 weeks before, during and 1 day after 3 days of cycling (Nieman et al., 2008). Interestingly, in recreational cyclists, the administration of a butyrylated high-amylose maize starch (HAMSB) for 28 days significantly increased the fecal levels of the SCFAs butyrate and propionate and the amount of *Faecalibacterium prausnitzii*, when compared with those of the groups administered a low amylose maize starch dose. Since *F. prausnitzii* exerts considerable mucosal antiinflammatory effects and the health effects of SCFAs are well described, the authors suggested that HAMSB could improve the gut health of healthy active individuals compared with those who consume diets rich in refined starch, although the effects of this prebiotic on athletic performance were not evaluated (West et al., 2013). Regarding polyphenols, the supplementation of a soy protein complex containing blueberry and green tea polyphenols for 17 days increased the blood levels of metabolites unique to the polyphenol metabolism of gut bacteria, although neither treatment decreased the recovery speed or ameliorated the physiological stress associated with 3 days of intensified running in marathon and half-marathon runners (Nieman et al., 2013b). As far as we know, there are no RCTs in the literature that examine the effect of polyphenols on athletic performance on changes in GM and gut-derived metabolites. Altogether, the results indicate that the effects of prebiotics and polyphenols on athletic performance are in a very preliminary stage of the investigation and further RCTs are needed to describe their effects and to elucidate whether their effects are partly driven by changes in GM and microbial-derived metabolites.

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CHAPTER TWENTY

The future of genetically based nutritional and pharmacological ergogenic aids in sport

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20.1 Introduction

Sport and exercise performance are significantly influenced by nutrition, yet individuals respond differently to the same foods, nutrients, and supplements consumed. Supplements are often used to optimize body composition and enhance performance, with usage rates of 37%–93% among elite athletes (Knapik et al., 2016; Giannopoulou et al., 2013). Athletic performance variations among athletes appear to be partly due to small contributions of hundreds of genes (Cieszczyk et al., 2016; Pitsiladis et al., 2013). Understanding the ways in which we can use ergogenic aids or supplements as a means of optimizing sport and exercise performance by aligning recommendations to known genetic variations, is of keen interest. The objective of this chapter is to examine the scientific evidence on specific ergogenic aids whereby genetic variants have been shown to or may have the potential to modify individual responses related to athletic performance.

20.1.1 Caffeine

20.1.1.1 Use and mechanism of action

Caffeine has become ubiquitous in the sporting world, where there is intense interest in better understanding the impact of caffeine on various types of exercise and sport performance in the individual. Accordingly, caffeine has dominated the ergogenic aids and sport supplements research domain over the past several decades (Higgins et al., 2016; Doherty and Smith, 2005; Ganio et al., 2009). Along with increases in coffee intakes, athletes have also increased their consumption of other caffeine-containing products, such as energy drinks (Rybak et al., 2015; Bailey et al., 2014), "preworkout supplements," chewing gum, energy gels and chews, aerosols, and many other novel caffeinated food products (Wickham and Spriet, 2018). To date, the preponderance of caffeine and exercise performance literature has utilized anhydrous caffeine (capsule) (Goldstein et al., 2010;

Sports, Exercise, and Nutritional Genomics
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Pasman et al., 1995; Lieberman et al., 2002; Graham and Spriet, 1991, 1995; Spriet et al., 1992; McNaughton et al., 2008) for ease in standardizing doses and creating the placebo form of the supplement. This is also the form that is used by most high-performance athletes in structured training programs, where recommendations are based on both research and on-field experience.

The strongest evidence suggests that the main target for caffeine's influence is the central nervous system (CNS), which is now widely accepted as the main mechanism by which caffeine alters mental and physical performance (Meeusen et al., 2013). Caffeine is believed to exert its effects on the CNS via the antagonism of adenosine receptors, leading to increases in neurotransmitter release, motor unit firing rates, and pain suppression (Black et al., 2015; Motl et al., 2003, 2006; Gliottoni et al., 2009; Gonglach et al., 2016). Acute caffeine ingestion has been shown to alter ratings of perceived exertion, where effort may be greater under caffeine conditions, yet it is not perceived as such (Doherty and Smith, 2005; Killen et al., 2013; Hadjicharalambous et al., 2006; Demura et al., 2007).

20.1.1.2 Genetic variation and caffeine response

Numerous studies have investigated the effect of supplemental caffeine on exercise performance, but there is considerable interindividual variability in the magnitude of these effects (Ganio et al., 2009; Higgins et al., 2016; Graham and Spriet, 1991) or in the lack of an effect (Hunter et al., 2002; Roelands et al., 2011) when compared to placebo. Most studies on caffeine and performance do not explore the basis for the interindividual variation in response, which has been well-documented in several studies (Graham and Spriet, 1991; Doherty et al., 2002; Wiles et al., 2006; Roelands et al., 2011; Jenkins et al., 2008). For example, Jenkins et al. (2008) examined the effects of caffeine on exercise performance in 13 cyclists, and the interindividual range for performance change with caffeine at 1, 2, or 3 mg/kg compared with placebo was −7.9%–17.8%. Although 11 of 13 cyclists benefited from caffeine (3 mg/kg dose), two "nonresponders" did not. The authors noted that "the mean performance outcome did not reach statistical significance due to these two "nonresponders" who strongly influenced the mean and SEM, in addition to 8 of the 13 subjects who performed worse on at least one caffeine condition versus placebo. Similarly, Paton et al. (2015) found that caffeinated (∼3–4 mg/kg) chewing gum improved overall performance in a group of 20 male and female cyclists, but only 13 (65%) of the cyclists were considered 'positive responders' while 5 (20%) experienced "negative" responses and the remaining 2 (15%) experienced no observable effect on cycling performance. The authors speculated that this variation in response may be related to differences in the rate of caffeine metabolism or absorption between individuals (Paton et al., 2015). Genetics are known to affect the rate of caffeine metabolism as described below. Due to infrequent reporting of individual data it is difficult to determine the extent to which variation in responses may be occurring. In the field of

nutrigenomics, caffeine is the most widely researched compound with several randomized controlled trials investigating the modifying effects of genetic variation on exercise performance (Pataky et al., 2016; Guest et al., 2018; Rahimi, 2018; Womack et al., 2012). These interindividual differences appear to be partly due to variations in genes such as *CYP1A2* which is associated with caffeine metabolism and response (Yang et al., 2010).

Over 95% of caffeine is metabolized by the CYP1A2 enzyme, which is encoded by the *CYP1A2* gene (Begas et al., 2007). The −163A > C (rs762551) single nucleotide polymorphism (SNP) has been shown to alter CYP1A2 enzyme inducibility and activity (Ghotbi et al., 2007; Djordjevic et al., 2008), and has been used to identify and categorize individuals as "fast" or "slow," or sometimes "ultra-slow" (Guest et al., 2018) metabolizers of caffeine. It has been shown that individuals with the AC or CC genotype (slow metabolizers) have an elevated risk of myocardial infarction (Cornelis et al., 2006), hypertension and elevated blood pressure (Palatini et al., 2009; Soares et al., 2018), and prediabetes (Palatini et al., 2015), with increasing caffeinated coffee consumption, whereas those with the AA genotype show no such risk. Additionally, regular physical activity appears to attenuate the increase in blood pressure induced by caffeine ingestion; however, it seems this is only beneficial for individuals with the AA genotype (Soares et al., 2018).

The largest caffeine and exercise study to date (Guest et al., 2018) examined the effects of caffeine and *CYP1A2* genotype on 10-km cycling time trial (TT) performance in competitive male athletes (both endurance and power sports) after ingestion of caffeine at 0, 2 (low dose), or 4 mg (moderate dose) per kg body mass. There was a 3% improvement in TT cycling time in the moderate dose in all subjects, which is consistent with previous studies using similar doses (Ganio et al., 2009; Desbrow et al., 2012). However, there was a significant caffeine-gene interaction; improvements in performance were seen at both caffeine doses, but only in those with the AA genotype who are 'fast metabolizers' of caffeine. In that group, the 6.8% improvement in cycling time was observed at 4 mg/kg, which is greater than the 2%–4% mean improvement seen in several other cycling TT studies, using similar doses (Ganio et al., 2009; Desbrow et al., 2012; Skinner et al., 2013; Saunders et al., 2017; Jenkins et al., 2008; Graham-Paulson et al., 2016; Bortolotti et al., 2014). Among those with the CC genotype, "slow metabolizers," 4 mg/kg caffeine impaired performance by 13.7%, and in those with the AC genotype there was no effect of either dose (Guest et al., 2018). The findings are consistent with a previous study (Womack et al., 2012) which observed a caffeine-gene interaction and improved TT cycling performance with caffeine only in those with the AA genotype.

The effects of genotype on performance are most prominent during training or competition of longer duration or an accumulation of fatigue, that is, muscular endurance, where caffeine appears to provide its greatest benefits, and where the adverse effects

to slow metabolizers are more likely to manifest (Doherty and Smith, 2004; Shen et al., 2019). In a study of basketball performance in elite players, caffeine improved repeated jumps (muscular endurance), but only in those with the AA genotype (Puente et al., 2018). Similarly, in a crossover design of 30 resistance-trained men, caffeine ingestion resulted in a higher number of repetitions per sets and for total repetitions in three resistance exercises combined, which resulted in a greater volume of work compared to placebo conditions, but only in those with the *CYP1A2* AA genotype (Rahimi, 2018). There appears to be growing support for the role of *CYP1A2* in modifying the effects of caffeine ingestion on aerobic or muscular endurance-type exercise. From a practical perspective, this helps to determine which athletes are most likely to benefit from caffeine and should experiment with it use. In contrast genetic studies also reveal those who may be less likely to benefit, and may possibly perform worse (Guest et al., 2018). Therefore, caffeine is one of the few ergogenic aids that is considered both a pharmacological, as anhydrous caffeine, and a nutritional, as coffee, tea, and botanical, supplement whose actions are modified through genetic variation. This research provides a template for the study of other ergogenic aids.

20.1.2 Buffering agents

20.1.2.1 Lactate transport

Lactate is the end product of anaerobic glycolysis and is constantly produced from pyruvate via the enzyme lactate dehydrogenase (LDH) during normal metabolism (Gladden, 2004). During high-intensity exercise muscular fatigue develops due to the accumulation of lactate, as the rate of lactate production exceeds the rate of removal (Finsterer, 2012). Blood lactate removal is governed by a number of factors, including monocarboxylate transporters (MCTs), concentration and isoform of LDH, and oxidative capacity of tissues (Bonen, 2001).

Most of the membrane lactate transport (in symport with a proton) is mediated by monocarboxylate transporters (MCT1 and MCT4), with the MCT1 being the predominant isoform in muscles (Fishbein et al., 2002; Pilegaard et al., 1999). The MCT1 transporter plays a relevant role in the intracellular pH homeostasis (Cupeiro et al., 2016). This is imperative after high-intensity exercise, where higher MCT1 content is associated with blood lactate concentration and blood lactate removal at rest (Green et al., 2002) and more importantly during active recovery (Cupeiro et al., 2016). Specifically, oxidative skeletal muscle contraction at submaximal intensities uses lactate as a respiratory fuel, and will be the primary consumer in clearing lactate from the blood stream (Gladden, 2004; Baldari et al., 2004). The rapid transport of lactate across the plasma membrane is fundamentally important to maintain work output at a given intensity for as long as possible, and for expedited recovery within the same training session or competitive event (Adeva-Andany et al., 2014). Active recovery is not only relevant after exercise cessation, but is also the most common situation during exercise protocols, such as

high-intensity interval training protocols (HIIT), repeated sprints and during intermittent team sports such as hockey, soccer, rugby, and basketball (Cupeiro et al., 2016; Macutkiewicz and Sunderland, 2011). Furthermore, optimizing recovery in rest periods or breaks such as those occurring in team sports, combat sports, ski racing, or medal rounds will impact subsequent performance (Chycki et al., 2018). In hockey, for example, there is an opportunity to reduce muscle lactate levels between periods (20 min), as well as between shifts (4–5 min) on the ice (Montgomery, 1988). Higher participation of MCT1 during these "recovery" periods, reflects the key role of MCT1 on high lactate transport rates (Skelton et al., 1998). Variations in MCT1 content and activity inevitably determine performance during higher-intensity exercise and sports (Halestrap and Price, 1999; Baker et al., 1998).

20.1.2.2 Genetic Variation in MCT1/SLC16A1

Variation in the *MCT1* gene (*SLC16A1*; rs1049434) which encodes the MCT1 transporter may modify lactate clearance (Merezhinskaya et al., 2000). *SLC16A1* or *MCT1* polymorphism has been associated with lactate transport and sports performance (Massidda et al., 2018). Greater lactate reduction has been observed in the AA genotype compared to TT genotype during high intensity exercise in one study (Cupeiro et al., 2010), with the opposite being observed, that is, greater lactate reduction in T-allele carriers in a similar study by the same group (Cupeiro et al., 2012). The authors concluded that the T allele induces higher lactate values in capillary blood but lower in venous blood, which reflect decreased lactate transport by MCT1 (Cupeiro et al., 2012, 2016). The *MCT1* A allele has also been significantly higher in endurance-oriented rowers (Fedotovskaya et al., 2014) and forward football player status (Massidda et al., 2018) compared to controls. However, in another study *MCT1* T allele was more prevalent in anaerobic (sprint/power) athletes than in both controls and endurance athletes (Sawczuk et al., 2015). The authors suggest that high lactate levels may induce the expression of muscle hypertrophy associated genes (i.e., those encoding mTOR, growth hormone, etc.), as increased lactate levels have been found to be associated with anabolic hormones and muscle hypertrophy (Bonen et al., 1998). This may assist in the accretion of lean body mass and enhanced sprint/power performance. These findings may be sport specific, as the AA genotype of the *MCT1* was found to be more prevalent in wrestlers compared with controls and is associated with lower blood lactate concentrations after 30-s Wingate test and during intermittent sprint tests in Japanese wrestlers (Kikuchi et al., 2017). Although the preponderance of evidence suggests that the greatest levels of lactate reduction has been observed in the AA genotype of the *MCT1* (Massidda et al., 2018; Kikuchi et al., 2017; Cupeiro et al., 2010, 2012), the combined results of several studies remain equivocal. Future studies are encouraged to replicate findings in other elite athlete cohorts, but presently it appears that athletes carrying the T-allele in *MCT1* may be most benefitted by buffering agents.

20.1.2.3 Beta-alanine, sodium bicarbonate, and other buffering agents

As mentioned, many sporting disciplines involve high-intensity performances at or nearing anaerobic capacity, whether continuous over several minutes as in many running, cycling, and swimming events or intermittent as in many team sports. Considering the high correlation of exercise-induced acidosis and fatigue, the ingestion of potential buffering agents have been suggested to attenuate metabolic acidosis and improve high-intensity sport performance (Lancha Junior et al., 2015). Sodium bicarbonate, sodium citrate, and sodium or calcium lactate, can all result in increased circulating bicarbonate and have all been shown to improve exercise capacity and performance under various circumstances (Lancha Junior et al., 2015). Most empirical data support the benefits of bicarbonates or related substances on exercise performance of different type, duration, and intensity (Miller et al., 2016; Krustrup et al., 2015; Tobias et al., 2013), however there appears to be some inconsistencies as other reports show no benefits (Edge et al., 2006; Siegler and Gleadall-Siddall, 2010).

Genetic variation in *MCT1* may in part play a role in these inconsistencies, and there is a need to design future trials that include stratification by *MCT1* genotype and buffering agent interventions. These studies should aim to identify the potential for gene-buffer interactions that may modify lactate clearance rates and resultant high-intensity exercise performance. The implications include the ability, through supplementation, to alleviate symptoms associated with impaired lactate clearance based on genotype. Athletes at risk may benefit from personalized recommendations for buffering agent use to enhance sport and exercise performance.

20.1.3 Carbohydrate supplements

Carbohydrates (CHO) provide the most important fuel source for the brain and muscle during exercise (Thomas et al., 2016). In addition to fueling the body, provision of CHOs to maintain blood glucose during exercise, lowers stress hormones including cortisol, and has been shown to minimizing postexercise immune dysfunction (Maughan et al., 2018; Bermon et al., 2017). CHO supplementation can also enhance performance through improving the bioavailability of other ergogenic aids such as creatine (Maughan et al., 2018). The body can store up to 740 g (~3000 kcal) of CHOs as glycogen in the muscle (80%) and liver (10%–15%) (Maunder et al., 2018; Gonzalez et al., 2016). Compared to endogenous fat stores (>100,000 kcal for a 75 kg person with 15% body fat), however, CHO stores are small and, therefore, if CHOs are not supplemented during exercise can adversely impact performance among athletes engaged in moderate to high intensity (~50%–90% VO_2 max) activity lasting >45 min in duration (Gonzalez et al., 2016). Numerous studies have shown that compared to placebo, ingestion of CHOs has been shown to prevent hypoglycemia, as well as maintain CHO oxidation rates and increase endurance capacity during prolonged exercise (>2 h) (Jeukendrup, 2014; King et al., 2018)—has multiple references.

In the past, recommendations have been to ingest 30–60 g of CHO per hour during endurance exercise (with >1 h duration) (King et al., 2018; Jeukendrup, 2014) This was based on the belief that ingested CHOs could only be oxidized at a maximal rate of 1 g/min (60 g/h) during exercise (Jeukendrup, 2014). However, multiple lines of evidence have supported the hypothesis that CHO oxidation rates can be higher and that the rate-limiting factor for maximal CHO oxidation is related to saturation of the glucose-specific transporter, SGLT1, in the brush border of the gut (Jeukendrup, 2017). When glucose is ingested at 60–70 g/h, exogenous CHO oxidation peaks around 60 g/h (Jeukendrup, 2017).Similarly oxidation rates do not increase when consuming CHOs at 144 or 180 g/h (Jeukendrup, 2017). However, a number of studies have shown higher CHO oxidation rates and improved performance when given a CHO supplement that contains both glucose (or maltodextrin, which is broken down to glucose) and fructose over a supplement that only contains glucose (Jeukendrup, 2014; King et al., 2019). Since fructose is transported by GLUT5 and does not compete for transport with glucose via SGLT1, providing multiple transportable CHOs appears to overcome the limitation of relying on only the SGLT1 transporter for transporting glucose across the brush border of the intestinal lumen (Orru et al., 2018).

In a double-blind randomized crossover study comparing placebo to four CHO-containing solution drinks: two contained only glucose, a low 60 g/h and a high 75 g/h glucose drink and two contained a combination of glucose:fructose providing 90 g (60:30 g) or 112.5 g (75:37.5 g) total CHO (at 2:1 glucose:fructose ratio) (King et al., 2018). This study showed that the 90 g glucose:fructose combination resulted in improved performance and higher exogenous CHO oxidation rates compared to the drinks containing only glucose (King et al., 2018). Although absolute muscle glycogen oxidation was not significantly different between the two combination drinks, the drink providing 112.5 g glucose:fructose combination adversely impacted the use of endogenous CHO stores from muscle glycogen for oxidation (King et al., 2018). To determine the more optimal dose of total CHO supplementation, a follow-up study of 11 male experienced cyclists cycled for 3 h (at 60% VO_2 max), followed by a 30-min time-trial and compared three glucose:fructose (2:1 ratio) solution drinks providing 80, 90, and 100 g/h with a placebo drink (King et al., 2019). This study showed that the 90 g/h dose was associated with improved performance and that at CHO provision at levels of 100 g/h is associated with use of endogenous CHO stores from muscle glycogen for oxidation (King et al., 2019). Interestingly, the study by King et al. reports that although overall the 90 g CHO appears most well tolerated and beneficial in terms of performance, 3 out the 11 (27%) participants did not show optimal performance when consuming 90 g CHO (King et al., 2019). Differences in monosaccharaide transport abundance and/or function may explain the different response among this subsample of the participants.

A recent study by Seidelmann et al. reports that three variants in the SGLT1 exome portion of the gene are associated with significantly reduced 2-h glucose levels after an

oral glucose challenge in two European populations ($n = 5687$; and $n = 6784$) and approached significance in a third African American sample ($n = 2791$). The three non-synonymous genetic variants were reported to be in strong linkage disequilibrium and together comprise a risk haplotype with a minor allele frequency of 6.7% (Seidelmann et al., 2018). Although they did not directly measure glucose absorption rates, an extreme and rare, autosomal recessive condition, resulting from a complete loss-of-function mutation in the SGLT1 gene results in impaired glucose transport and cause glucose-galactose malabsorption which can lead to neonatal death from severe diarrhea unless glucose and galactose are removed from the diet (Seidelmann et al., 2018; Lam et al., 1999). In addition, a previous study has shown that the three SGLT1 variants have reduced glucose transport activity when expressed in oocytes (Martin et al., 1996). Therefore, testing SGLT1 haplotype by glucose ingestion on CHO oxidation rates and performance can determine whether those with reduced SGLT1 activity may benefit more from using a multiple transportable CHO approach and/or help fine-tune the optimal glucose:fructose ratio. Indeed, there is evidence that the optimal glucose:fructose dose ratio may be closer to a 1:1 ratio rather than a 2:1 ratio (Rowlands and Houltham, 2017) and variability across studies may be related to differences in monosaccharide transport efficiencies.

Historically, CHO supplements started out in the form of sport beverages with Gatorade first developed in 1965 by a team of researchers at the University of Florida, to be used by the school's football team and contained glucose as the sole CHO source (Gatorade). Today, CHO supplement options have greatly expanded to include commercially available sport drinks containing multiple transportable CHO sources as well as gels that also range in fructose content (Zhang et al., 2015) and glucose:fructose ratios ranging from 1.2:1 to 1:1, to >3:1 glucose-to-fructose ratio (Wilson et al., 2015). The study by Wilson et al. interestingly reports that although over half the triathletes were aware of the recommendations to consume multiple transportable CHOs, the median glucose-to-fructose ratio consumed over the race was closer to 3:1 than the recommended 1:1 (Wilson et al., 2015; Rowlands and Houltham, 2017). A more tailored approach to nutritional supplement recommendations using genetics to guide CHO targets may offer an ergogenic advantage to athletes. Development of commercial personalized sports drinks and other CHO supplement formulations such as gels and chews, based on genotype, would align with these goals (Nielsen and El-Sohemy, 2012).

20.1.4 Salt-electrolytes

Sodium is an essential mineral that maintains normal cellular homeostasis required for muscle and nerve cell excitability and is needed for the regulation of fluid and electrolyte balance and blood pressure (Strazzullo and Leclercq, 2014). Half of the sodium in the body is located in the extracellular fluid at a concentration of 135–145 mmol/L,

approximately 10% is found in intracellular fluid at a concentration of ~10 mmol/L, and about 40% is found in the skeleton (Strazzullo and Leclercq, 2014). In addition to water, athletes lose a considerable amount of sodium in sweat (Thomas et al., 2016). Given that sodium is the principal cation found in extracellular fluid, it is essential to replace both water and sodium lost during exercise to prevent the rise in serum osmolality and restore plasma volume (Shirreffs and Sawka, 2011). Importantly, fluid loss can negatively impact cognitive function and aerobic exercise performances at levels when body weight loss is >2% (Thomas et al., 2016; Casa et al., 2005). Therefore, hydration and maintenance of plasma volume is an important factor for optimal performance, especially in long-duration events (>90 min) taking place in warm climates (Shirreffs and Sawka, 2011). Athletes who overhydrate with water or hypotonic fluids to prevent dehydration can inadvertently put themselves at risk of exercise-associated hyponatremia (EAH), a condition characterized by sodium concentration < 135 mmol/L (Shirreffs and Sawka, 2011). EAH can result in serious complications, including encephalopathy and has been documented to occur in up to 51% of athletes competing in endurance events 26,237,602 (Urso et al., 2014), making it one of the most common life-threatening disorder impacting endurance athletes (Del Coso et al., 2016b). Although overhydrating is the primary risk factor for EAH, higher loss of sodium through sweat is proposed to be another mechanism associated with EAH (Thomas et al., 2016; Hew-Butler et al., 2017).

The 2005 American College of Sports Medicine recommends consuming snacks and fluids containing sodium to help reduce the risk of EAH and to treat muscle cramping (beverages containing 50–100 mmol/L sodium chloride salt) (American College of Sports Medicine et al., 2007). The recent 2016 Joint Position statement for Nutrition and Athletic Performance from the American College of Sports Medicine together with the Academy Of Nutrition And Dietetics and the Dietitians of Canada recommendations include consumption of sodium in fluids and foods, *prior* to exercise, to aid with fluid retention (Shirreffs and Sawka, 2011). In addition, sodium consumption is indicated *during* exercise when large sweat sodium losses occur, either from high sweat rates (>1.2 L/h), among individuals who experience "salty sweat," or when exercise duration is prolonged (> 2 h) (Shirreffs and Sawka, 2011). Finally, to help retain fluids and plasma volume, after exercise, the Joint Position Statement advises to rehydrate with water and sodium at a modest rate that minimizes diuresis (Shirreffs and Sawka, 2011). Despite recommendations to include sodium-containing fluids such as sport drinks, studies have reported mixed findings for the prevention of low sodium levels and muscle cramping (Lewis et al., 2014; Hoffman and Stuempfle, 2015; Hew-Butler et al., 2015, 2017; Casa et al., 2005). The discrepancy in findings could be related to a number of factors including differences in intensity and duration of activity across studies, environmental temperatures, study design as well as differences in individual needs for sodium to replace sodium losses (Del Coso et al., 2016a; Lara et al., 2016; Hoffman et al., 2015). Indeed, sodium lost in sweat has been shown to vary greatly across individuals, ranging from 7.0

to 95.5 mmol/L sodium losses in 157 experienced marathon runners (Lara et al., 2016). Therefore, underlying genetic variations in genes regulating sodium loss in sweat may play a role in impacting different sodium requirements for the prevention of low circulating sodium levels and muscle cramping.

Several studies have now examined the role of the CFTR gene as a biologically plausible candidate explaining the marked interindividual variability of sodium lost in sweat (Brown et al., 2011; Lewis et al., 2014; Del Coso et al., 2016b). CFTR encodes the cystic fibrosis transmembrane protein, which is responsible for sodium reabsorption in the sweat glands by its interaction with the epithelial sodium channel (ENaC) (Saint-Criq and Gray, 2017). Indeed, a hallmark symptom of cystic fibrosis is abnormal production of salty sweat (Saint-Criq and Gray, 2017). Two earlier studies examined 31+ variants known to be associated with cystic fibrosis, including the DeltaF508 cystic fibrosis-causing variant, to determine whether carrying a mutation in the CFTR gene is associated with EAH (Lewis et al., 2014) or risk of being classified as an exceptionally "salty sweater" (sweat sodium concentration >70 mmol/L) (Brown et al., 2011). The study by Lewis et al., examined ultra-marathon runners, and although 16% of those who completed the race and provided an end-of-race blood draw were classified as experiencing EAH, none of the runners were carriers of any of the 31 CFTR mutations (Lewis et al., 2014). The authors speculate that the inclusion criteria for the study which measured outcomes on only those who completed the 161-km marathon can introduce selection bias given the intensity of participating in such a race can possibly eliminate those who may be carriers of CFTR mutations (Lewis et al., 2014). Similarly the study by Brown et al., examined six healthy, extreme salty sweaters, and also report that none were carriers of the disease-causing CFTR gene mutations (Brown et al., 2011). The study by Brown et al., however, report lower expression of CFTR associated with being a salty sweater (Brown et al., 2011). The latest study by Del Coso et al. took a slightly different approach and examined gene variants that are not disease causing, a tract of polyT (IVS8-Tn) with allelic three variants: 5T, 7T, and 9T (Del Coso et al., 2016b). In their study examining 51 marathon completers, they observed that those who were 7T/7T homozygotes had higher concentrations of sodium lost in sweat (42.2 ± 21.6 vs. 29 ± 24.7 mmol/L) and a greater sodium imbalance (the sodium lost in sweat exceeded the sodium replaced by fluids) compared to those with the 7T/9T genotype (Del Coso et al., 2016b). The increased sodium loss observed was not associated with differences in circulating sodium levels (Del Coso et al., 2016b). However, the authors did not test for a gene-sodium intake interaction which may have shed light on whether those with the 7T/7T genotype who have lower sodium replacement levels from foods and/or fluids have lower levels of circulating sodium at the end of the race (Del Coso et al., 2016b).

Given that hypohydration can impact performance, but overhydrating can increase the risk of EAH, a fine balance is needed and a personalized approach can offer an optimal strategy for athletes to restore fluid losses while maintaining circulating sodium levels

(Shirreffs and Sawka, 2011). Personalized strategies proposed include assessment of urine properties before exercise, change in body mass that occurs during exercise after accounting for fluid intake, measured urinary losses, and sodium content of sweat (Maughan and Shirreffs, 2008). This type of individualized approach would require cumbersome data collection. Genetics offers an alternative strategy for individualizing sodium needs. First, studies that use genetics can help identify individuals who are at higher risk of greater salt loss in sweat or lower circulating sodium levels and who may therefore need higher sodium supplementation for prolonged exercise. To further fine-tune personalized sodium recommendations, studies taking into account the way these genes modify the way our body responds to sodium intakes by examining gene-diet interactions can identify the level of sodium needed among individuals at risk. Indeed, in the study of 157 marathon runners, 20% were reported to have sodium losses >60 mmol/L, and therefore a one-size-fits all recommendation (Murray, 2007) can underestimate the need for salt among 1 in 5 endurance athletes who are salty sweaters (Lara et al., 2016). Compared to plasma sodium levels, sport drinks are hypotonic (ranging between 10 and 38 mmol/L), and therefore excessive consumption of sodium-containing beverages can render useless in maintaining circulating sodium concentrations and preventing EAH (Hew-Butler et al., 2015, 2017). The use of salt supplements (in addition to sports drinks) offers an additional strategy for endurance athletes to compensate for the loss of salt especially in hot environments (Del Coso et al., 2016a). In a randomized control trial including 26 experienced triathletes randomized to a salt supplement group (2580 mg of sodium, 3979 mg of chloride, 756 mg of potassium, and 132 mg of magnesium) or control (cellulose), total race time was lower in the salt supplement group (Del Coso et al., 2016a). Both postrace serum levels of sodium and chloride were significantly higher in the salt supplement group compared to the control group (Del Coso et al., 2016a). The authors noted, however, that since salt supplementation was not individualized, the percentage of electrolytes replaced (compared to the electrolyte losses) was not equal for all the triathletes in the experimental salt supplementation group. Future studies need to consider how genetic variation in genes such as CFTR and other candidates involved in regulating sodium lost in sweat and/or genes involved in sensing blood osmolality, such as TRMP4 (Tian et al., 2009), can provide insight into ways in which sodium provisions may differ across athletes participating in ultra-endurance sport. By measuring intakes of sodium containing beverages, foods, and supplements while incorporating genetic information by testing how certain genetic variants can modify the relationship between sodium intakes on sport performance, sodium sweat losses, and circulating sodium levels, sport scientists will be able to begin mapping out a more personalized level of sodium needed to maintain circulating sodium levels and performance, while also taking into consideration limiting unwanted side effects such as sodium-sensitive hypertension (Hew-Butler et al., 2017) and gastrointestinal discomfort (Del Coso et al., 2016a).

20.1.5 Ketone bodies (KB)

The therapeutic effects of ketosis for disorders such as epilepsy have been studied for decades, but in recent years, public interest in ketosis for sports performance has risen (Egan and D'Agostino, 2016). Research on the use of KB as an ergogenic aid remains limited, but a few studies conducted in athletes within the last few years hint at the potential benefits of these metabolites as supplements for highly trained individuals engaging in competition (Cox et al., 2016; Egan and D'Agostino, 2016; Holdsworth et al., 2017; Leckey et al., 2017; Evans et al., 2017, 2018; Evans and Egan, 2018; Myette-Cote et al., 2018; O'Malley et al., 2017; Scott et al., 2019; Shaw et al., 2019). Nevertheless, the results are inconsistent and many questions remain not only about the type, dose, and mode of delivery of KBs for performance improvements, but also about their effects on different individuals. The present section provides a brief summary of the available knowledge on KB supplementation for athletic performance and highlights opportunities for research on genetic variation to help explain the inconsistencies in response across studies.

20.1.5.1 Ketone body metabolism

Ketosis can be achieved endogenously through dietary means, including fasting or very low-CHO, high-fat ketogenic diets (Evans et al., 2017). The process likely evolved as a physiological adaptation to periods of food scarcity, given that KBs act as alternate sources of energy when CHO reserves are low (Egan and D'Agostino, 2016). KBs, including the ketones acetoacetate (AcAc) and acetone, and the AcAc-derived β-hydroxybutyrate (BHB), are produced by the liver in the context of depleted hepatic glycogen (Sansone et al., 2018). As plasma glucose and insulin drop, lipolysis in adipose tissue releases free fatty acids (FFA) into the circulation, and these serve as the primary basis for KB formation (Sansone et al., 2018). KBs are readily utilized as a substrate by the brain, which cannot draw energy from FFAs (Egan and D'Agostino, 2016). In brief, the generation of KBs (ketogenesis) involves FFA entry into liver mitochondria, beta-oxidation into acetyl-CoA, and conversion into AcAc via a series of reactions mediated by the enzymes Ac-CoA acetyltransferase (ACAT), hydroxymethylglutaryl CoA synthase (HMGCS), and HMG-CoA lyase (HMGCL) (Evans et al., 2017). The resulting AcAc can enter the circulation, but the majority is reduced into BHB by 3-hydroxybutyrate dehydrogenase (BDH). BHB is carried into the bloodstream by the solute ligand carrier protein 16A (SLC16A), which is a type of MCT (Evans et al., 2017). Unlike AcAc and BHB, acetone results from spontaneous decarboxylation of AcAc and its role as a fuel substrate is negligible (Sansone et al., 2018). For BHB and, to a limited extent, AcAc to act as alternate energy substrates, they must enter the mitochondrial matrix of extra-hepatic tissues through MCT-mediated transport (Evans et al., 2017). In the process of ketolysis, BHB undergoes reoxidation into AcAc via BDH, and AcAc undergoes conversion into

two molecules of Ac-CoA through sequential catalysis mediated by succinyl-CoA:3-oxoacid CoA transferase (OXCT) and ACAT. Ac-CoA then enters the tricarboxylic acid (TCA) cycle (Evans et al., 2017). KBs represent a more efficient energy source than CHO, with BHB releasing 13 ATP/mol per C_2 unit, as compared to 12.67 for glucose and 10 for pyruvate (Shaw et al., 2019).

Skeletal muscle has a strong affinity for KBs, but under standard feeding conditions KB concentrations in the circulation are <0.1 mM and their energy contributions are minimal (Evans et al., 2017). These concentrations rise to 0.2 mM after an overnight fast, when KBs represent around 10% of the contribution to skeletal muscle energy. Plasma concentrations rise steadily to approximately 1–2 mM at 72 h of fasting, at which point KBs contribute as much as 50% of energy to muscle tissues. However, longer fasts of up to 3 weeks or more are characterized by a decrease in the energy contribution of KBs to muscle metabolism from 50% down to 15%, suggesting that skeletal muscle becomes saturated at plasma concentrations above 2 mM (Evans et al., 2017).

20.1.5.2 Ketosis and exercise

The evidence for the potential beneficial effects of KBs on athletic performance remains limited, but intriguing. Early experiments conducted in rodents suggest that exercise-trained muscle tissue can use KBs for energy better than untrained muscle (Winder et al., 1975). In humans, expression of the transport protein MCT1is higher after muscle training and correlates positively with the intensity of the training (Thomas et al., 2012). Interestingly, expression of enzymes involved in KB transport and metabolism is much higher in type I than type II muscle fibers and it is correlated with muscle oxidative capacity (Bonen, 2001). Taken together, these studies suggest that highly trained individuals with a higher relative abundance of type I muscle fibers and high oxidative capacity are more likely to benefit from high-circulating KB concentrations. As such, the available work on KB supplementation for athletic performance thus far has focused on endurance sports (see below).

KBs appear to serve purposes other than acting as an energy substrate during CHO deprivation. Indeed, the presence of KBs seems to spare glucose use by muscle as well as lipolysis of fat stores (Cox et al., 2016; Leckey et al., 2017). Meanwhile, intramuscular lipolysis increases (Cox et al., 2016). Antiproteolytic effects of ketones have also been noted, with reduced muscle protein breakdown during exercise. Reductions in lactate production have been observed as well (Cox et al., 2016; Leckey et al., 2017; Evans and Egan, 2018). KBs have also been proposed to exert beneficial effects on oxidative stress and inflammation (Newman and Verdin, 2017), although recent work suggests potential proinflammatory effects of KBs (Neudorf et al., 2019). Overall, some of the available studies point to potential benefits of KBs on exercise through involvement in the preservation of more traditional fuel stores as well as potential effects on recovery. This limited, but suggestive, body of evidence has led some to attempt to harness the

potential of these metabolites for performance. However, the endogenous conditions under which KBs are generated necessitate nutritional protocols that are either impractical or downright deleterious for athletes engaging in intense training. Fasting is not advisable for these individuals due to the high energy demands of their exercise regimen, and ketogenic diets are likely too restrictive for long-term maintenance. Furthermore, these diets work by reducing CHO stores and leave little substrate available for glycolysis in type II muscle fibers, therefore reducing the ability of the athlete to execute movements involving sudden bursts of energy—which, while critical to power sports, also play a key role in the occasional sprints that happen during endurance racing.

20.1.5.3 Exogenous ketone body supplementation for athletic performance

Given the impracticality and potential detrimental effects on performance of ketosis achieved through dietary means, exogenous KB administration for athletes appears a suitable alternative, but to date, research on the effects of KBs administered as a supplement on performance remains limited. Until recently, work on the physiological effects of exogenous KBs in both animal models and humans used ketone salts, the latter being impractical as a vehicle for routine supplementation (Evans et al., 2017). Ketone salts are available commercially, but their effectiveness at increasing plasma concentrations of BHB is limited, and some research indicates that they elicit gastrointestinal discomfort and may lead to cation overload or acidosis (Veech, 2004). More recently, ketone esters (KEs) purported to lack the limitations of ketone salts have been developed, and some of these have been tested in athletes. The first study reporting on the effects of KEs on athletic performance was published in 2016 (Cox et al., 2016). Experiments were carried out in a randomized crossover design in a total of 39 overnight-fasted endurance athletes who were given a ketone monoester derived from BHB (dose 573 mg/kg body weight), either alone or in various combinations with CHOs and fat, before and/or during cycling workouts differing in length and relative intensity (Cox et al., 2016). The results of the study suggested that BHB was produced after KE supplementation (with plasma BHB concentrations during exercise at about 3.5 mM) and contributed approximately 16%–18% of energy during the workouts. Furthermore, exercise-induced increases in circulating lactate were 50% lower after a KE-containing beverage than an isocaloric CHO-containing drink. After consuming KEs, glycolysis decreased whereas intramuscular triglyceride used increased, with a concomitant sparing of glucose, and the authors observed a decrease in exercise-induced protein degradation. Importantly, consuming a drink consisting of 40% kcal from KEs and 60% CHO prior to a cycling TT resulted in a 2% improvement in performance, compared to an isocaloric beverage containing 100% CHO (Cox et al., 2016). Such an improvement is meaningful in the context of high-level competition, where minor gains can place an athlete ahead of other equally highly trained individuals. Subsequent work suggests that KE supplementation along with CHO after a bout of exercise results in increased muscle glycogen replenishing in

athletes, although the findings are inconsistent across studies (Holdsworth et al., 2017; Vandoorne et al., 2017). As a note of caution, in a recent study, supplementation with the same KE decreased blood pH while increasing BHB concentrations to approximately 3.5 mM (Dearlove et al., 2019) suggesting potential dangers of ketoacidosis in the context of KE supplementation,

Other research on KBs and athletic performance has yielded inconsistent results. Another randomized, crossover trial using KEs provided 10 professional cyclists with an AcAc-derived ketone diester supplement (Leckey et al., 2017). The athletes completed a 31-km TT simulating the 2017 World Road Championships course and followed a preexercise feeding and warmup protocol typical of such a competition. A KE-containing drink (250 mg/kg) or placebo was administered 30 min before and immediately prior to the warmup. Blood BHB increased to approximately 0.5 mM, although the authors reported discrepancies in the concentrations measured from serum versus capillary whole blood (Leckey et al., 2017). KE supplementation led to a 2% decrease in performance as measured by time to complete the trial, as well as power output. Nevertheless, in agreement with Cox et al. (2016), lower blood glucose and lactate concentrations were observed with KE supplementation as compared to placebo (Leckey et al., 2017). In a study conducted in healthy individuals who were not athletes (Myette-Cote et al., 2018), the same ketone monoester used by Cox et al. (2016) also led to a blunted glycemic response after an oral glucose tolerance test. Yet another study using the same ketone monoester was carried out in athletes engaging in the Loughborough Intermittent Shuttle test, which is a validated assessment of the physiological responses required in a soccer match (Evans and Egan, 2018). The athletes experienced elevations in BHB ranging from 1.5 to 2.6 mM, despite receiving 750 mg/kg of KE supplement, which was higher than the dose provided by Cox et al. (2016). A trend for longer running time to exhaustion was observed with the KE supplements, although it failed to reach statistical significance. In concordance with the previous studies, KE supplementation led to lower blood glucose and lower lactate concentrations (Evans and Egan, 2018). Taken together, these findings using different KEs in different populations suggest that the effects on blood glucose and lactate concentrations seen across studies may be robust. However, effects on performance appear to be inconsistent.

A number of studies have used exogenous KBs other than KEs in examining performance, as well as physiological responses potentially related to performance (Evans et al., 2017; O'Malley et al., 2017; Rodger et al., 2017). As mentioned earlier, ketone salts elicit lower BHB concentrations than KEs (typically in the 1 mM range), which may be insufficient to observe ergogenic benefits. Furthermore, they commonly lead to gastrointestinal distress that may also affect performance (Evans et al., 2017, 2018). Two recent studies (Scott et al., 2019; Shaw et al., 2019) used 1,3-butanediol, a component of 1,3-butanediol AcAc diester, and they found no improvements in performance during either a cycling or a TT; in one study the participants also reported significant

gastrointestinal distress (Shaw et al., 2019). In both studies, the BHB concentrations elicited by the supplement were approximately 1 mM, again raising the question of whether sufficient ketosis was achieved for ergogenic gains (Shaw et al., 2019; Scott et al., 2019). Consistent with previous studies using KEs, Scott et al. (2019) observed lower lactate concentrations at certain time points after supplementation.

20.1.5.4 Genetic variation and the response to exogenous ketone body supplementation

As highlighted by the studies above, the effects of KB supplementation on various aspects of performance appear to be variable. The inconsistencies across studies are partly a result of methodological differences including the use of different KB supplements at varying doses, as well as type, duration and intensity of the exercise challenge, degree of muscular glycogen depletion prior to the tests, etc. Interindividual genetic variation may also play a role in the response to KB supplements, but this potentially important confounding factor remains, to our knowledge, virtually unexplored. Among the studies cited in the previous section, every single one that presents individual responses in the published manuscripts shows differences in response across participants for specific outcomes (Cox et al., 2016; Leckey et al., 2017; Evans and Egan, 2018; Myette-Cote et al., 2018; O'Malley et al., 2017; Scott et al., 2019; Shaw et al., 2019). Further research is necessary to elucidate the potential contributions of genetic variation to the observed differences. Enzymes involved in KB metabolism, such as BDH, OXCT, and ACAT, as well as the carrier protein SLC16A, represent potential candidate genes for future studies. Examining the relationship between variants in these and other genes and response to exogenous KBs could lead to a better understanding of the physiological effects of these supplements. Furthermore, understanding how individual athletes respond to KB supplementation could facilitate the development of personalized supplementation strategies to improve performance.

20.1.6 Vitamin C and collagen supplementation for connective tissue injuries

As a potent antioxidant, vitamin C plays a crucial part in the body's defense mechanisms against reactive oxygen species (ROS), production of which is elevated during athletic performance (Guest et al., 2019; Heaton et al., 2017). However, supplementation with vitamin C to manage oxidative stress has been found to interfere with exercise-induced metabolic adaptations and may result in worse performance (Guest et al., 2019; Heaton et al., 2017). As a result, recommendations for consumption of vitamin C as an antioxidant are based on intake from traditional dietary sources. However, in addition to its role as a reducing agent, vitamin C is involved in other physiological processes that are associated with athletic performance and recent research suggests certain beneficial effects of vitamin C supplementation, as discussed below.

20.1.6.1 Vitamin C and collagen formation

One of vitamin C's central roles is as a positive regulator of collagen synthesis (Heaton et al., 2017; Shaw et al., 2017; Dephillipo et al., 2018). Ascorbic acid, the main circulating form of vitamin C, is a necessary cofactor in the hydroxylation of proline residues within procollagen, and hydroxyproline is required to stabilize the triple helix characteristic of collagen fibrils (Peterkofsky, 1991). In addition, vitamin C is involved in collagen crosslinking, which affects its mechanical function, through activation of lysyl oxidase and prolyl and lysyl hydroxylases (Shaw et al., 2017). Work conducted in pregnant rats has shown that vitamin C supplementation results in stronger collagen staining of the examined tissues (Findik et al., 2016), and systematic reviews on the role of vitamin C supplementation to ameliorate the pain associated with healing from wrist fractures (Aim et al., 2017) and collagen synthesis after musculoskeletal injuries (DePhillipo et al., 2018) have yielded suggestive results, notwithstanding the limited available evidence. As such, vitamin C may play a therapeutic role in connective tissue injuries, which are extremely common among athletes.

20.1.6.2 Vitamin C and collagen supplements for connective tissue health

Parallel to the evidence for benefits of vitamin C supplementation, use of collagen supplements for connective tissue injury prevention and repair has grown steadily among athletes. However, until recently, little evidence was available on the efficacy of collagen supplements or the potential need to consume them with vitamin C for beneficial effects. A study conducted in 2017 yielded insights directly relevant to this question (Shaw et al., 2017). The study was a randomized, double-blind, crossover intervention carried out in eight healthy young men who consumed a drink containing 48 mg vitamin C and either 5 or 15 g of gelatin or placebo, 1 h prior to each of three daily 6-min skipping sessions, for a total of 3 days (Shaw et al., 2017). The participants provided blood samples at various time points, from which the concentrations of amino acids enriched in collagen, as well as procollagen, were determined. In addition, the authors developed engineered ligaments in vitro and cultured them in serum from the participants to capture any collagen formation, as well as measures of ligament mechanics (Shaw et al., 2017). Overall, the results of the study indicated a dose-dependent increase in collagen production, with the supplements containing 15 g of gelatin resulting in the greatest benefit. While collagen production required supplementation with both vitamin C and gelatin, improvements in engineered ligament mechanics were noted even in those treated with placebo, suggesting that vitamin C, even in the absence of a collagen supplement, improved collagen crosslinking, and subsequent ligament function (Shaw et al., 2017). A more recent study from the same group determined the effects of providing a vitamin C-collagen supplement in the form of either gelatin, hydrolyzed collagen, or a combination of the two, with the dose consisting of 15 g of collagen and 48 mg of vitamin C. The vehicle was a beverage in the case of gelatin or hydrolyzed collagen, and a gummy in the

treatment where the two were combined. Participants again consumed the supplement 1 h prior to exercise, and changes in blood concentrations of procollagen and amino acids enriched in collagen were evaluated (Lis and Baar, 2019). The authors again noted increases in blood procollagen and collagen-enriched amino acids, although the observed trends were not statistically significant and the authors attributed this to a large degree of variation across participants (Lis and Baar, 2019). Taken together, these studies provide limited but interesting evidence that vitamin C supplementation, particularly along with collagen, may increase collagen synthesis and possibly improve ligament mechanics. This may result in decreased connective tissue injury risk among athletes, but more research is needed.

20.1.6.3 Genetic variation, vitamin C, and connective tissue injuries

The large interindividual variation noted in the study by Lis and Baar (2019) suggests that unaccounted factors may confound the observed results. One such unaccounted confounder is genetic variation across individuals. Indeed, previous research suggests that an insertion-deletion variant in the *GSTT-1* gene affects circulating levels of ascorbic acid (Cahill et al., 2009). *GSTT-1* encodes a glutathione S-transferase involved in reducing dehydroascorbic acid back to ascorbic acid. The gene has two alleles: *GSTT1**0 (nonfunctional) and *GSTT1**1(functional). In a cross-sectional study of adults aged 20–29 years, homozygosity for the *GSTT1**0 variant was associated with an increased risk of vitamin C deficiency (as determined by ascorbic acid serum concentrations) when vitamin C intake was below the RDA. By contrast, individuals who carried the *GSTT1**1 allele appeared to be protected from low ascorbic acid concentrations, even if their vitamin C intake was below the RDA (Cahill et al., 2009). This work highlights the importance of considering genetic variants that may modify the relationship between diet and outcomes.

To our knowledge, no studies have examined whether gene-diet interactions modify the relationship between vitamin C-collagen supplements and connective tissue injuries. We do, however, know that genetic variation affects risk of connective tissue injuries. For example, variation in *COL5A1* is associated with Achilles tendinopathy (September et al., 2009). The α 1 type V collagen (*COL5A1*) gene encodes the pro-α1(V) chain, a component of collagen V, which is a building block in connective tissue (September et al., 2009). This is just one of several genes involved in collagen formation. Variation in *COL5A1* and other collagen-related genes, together with variation in *GSTT1* and other genes involved in vitamin C metabolism, likely affects collagen formation and structure and, subsequently, the effects of vitamin C-collagen supplements on specific individuals. More research is needed to better understand how genetic variants affect athletes' responses to these supplements, so that targeted supplementation strategies may be developed to minimize injury risk and improve performance.

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Gatorade History

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CHAPTER TWENTY-ONE

Perspectives of personalized weight loss interventions based on exercise genomics, nutrigenetic, epigenetic, and metagenomic data in fitness and sport

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21.1 Introduction

The epidemic of obesity, together with its associated comorbidities, continues to spread globally (Sepúlveda and Murray, 2014) and, for this reason, there is an urgent need to develop more effective weight loss interventions (Celis-Morales et al., 2014). Genotype background and epigenetic signature play an important role in obesity's development (Campion et al., 2009; Willyard, 2014) and, more interestingly, in how individuals respond to different weight loss strategies.

Although the mechanisms that lead to weight loss are complex and multifactorial including behavior, environmental, inherited, and physiological factors (Kaila and Raman, 2008; Guyenet and Schwartz, 2012), the final determination for weight changes can be viewed most broadly by an energy imbalance resulted from energy overconsumption and low energy expenditure. Thus, a negative energy balance due to an energy restriction and regular exercise training can independently influence the degree of fat mass loss during weight loss interventions (Chaston et al., 2007).

In the line of this, evidence show that increased physical activity (PA) can reduce fat mass, increase muscle mass, and prevent weight gain (Bea et al., 2010; Teixeira et al., 2003). However, the magnitude of response to regular exercise training differs between individuals and, this interindividual variability is reflected in the concepts of trainability and translates to "subject-by-training interaction" (Arbab-Zadeh et al., 2014).

Nutrigenetics and exercise genomics represents the major anchoring pillars of personalized medicine and the pace of growth in exercise genomics research has increased substantially in the past several years (Sarzynski et al., 2016). The global goal of personalized medicine is to use each patient's unique genetic and environmental characteristics to

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design optimal strategies (Buford and Pahor, 2012). The purpose of this chapter is to summarize some of the latest developments in personalized interventions for weight loss gene-exercise interactions and discuss opportunities and challenges for future strategies.

21.2 Variability in response to exercise-induced weight loss interventions

Many studies have shown the role of exercise and dietary factors in weight control (Qi and Cho, 2008); however, considering the great individual variance, losing weight in response to exercise training may be more effective for some genotypes than others (Leońska-Duniec et al., 2016). Exercise genomics is a new science that aims define genetic and molecular markers that would make it possible to predict the benefits from a different exercise programs or a physically active lifestyle (Pérusse et al., 2013).

Recent studies have shown gene variant versus PA interactions (Li et al., 2010; Zou et al., 2015; Leońska-Duniec et al., 2017). Some authors demonstrated that physical active practice is associated with a 40% reduction of the genetic predisposition to obesity and emphasized the importance of exercise in the prevention of excess body weight (Li et al., 2010). Here, we described the most reliable candidate genes that are involved in energy balance pathways and body composition changes in response to exercise (Table 21.1).

21.2.1 FTO gene

Despite the exact function of fat mass and obesity associated gene (FTO) has not been well described, it is suggested a possible role in the control of energy homeostasis, with the FTO product acting as the primary regulator of body fat accumulation (Gerken et al., 2007). For this reason, FTO gene plays a role in the regulation of lipolysis (Zabena et al., 2009; Sébert et al., 2010) and has been wildly reported to be associated with fat mass and obesity in both adults and children. In this way, FTO rs9939609 polymorphism is most studied and may be associated with obesity and resistance to weight loss (Quan et al., 2015; Ursu et al., 2015).

Zou et al. (2015) investigated the association of FTO rs9939609 polymorphism on the effects of a diet and exercise intervention (low intensity aerobic physical exercise, six times a week and 2 h for 4 weeks) in obese children and adolescents. At the preintervention time, individuals with A risk allele (AA or AT genotype) had a higher BMI compared to those with TT genotype. Interestingly, biochemical and BMI parameters were significantly reduced after the intervention only in the AA or AT genotype, showing that the effect of exercise combined with diet intervention in Chinese children and adolescents is more effective for certain individuals dependent on the genotype. Another study with European adults found an association with FTO polymorphism and higher body weight, BMI, and waist circumference. However, individuals who

Table 21.1 Genes related to exercise-induced weight loss interventions

Gene name	Sigla	Author	SNP	Exercise protocol	Conclusion
Fat mass and obesity-associated gene	*FTO*	Zou et al. (2015)	rs9939609	Diet + aerobic exercise, 120 min, six times a week for 4 weeks	The effects of PA combined with dietary intervention on obesity were associated with the polymorphism in Chinese adolescents and children
		Celis-Morales et al. (2016)		Physical activity (3 to <6 METs)	PA attenuates the effect of FTO genotype on BMI and WC in European individuals
		Mitchell et al. (2010)	rs8050136	6 months of moderate PA, three–four sessions per week, 50% of the HR achieved at VO2 peak	No interaction between genotype and weight loss after physical exercise intervention in postmenopausal women
Melanocortin 4 receptor	*MC4R*	Leońska-Duniec et al. (2017)	rs17782313	12-week aerobic training, three sessions per week	No interaction was observed between MC4R C/T polymorphism and physical activity in Polish women
		Li et al. (2010)		PA was assessed using a self-administered questionnaire	Genetic predisposition to increase BMI is reduced by a PA lifestyle in European adults
		Zlatohlavek et al. (2013)		4 weeks of reduction in energy intake and exercise program of 5 exercise units per day, 50 min	BMI decrease in overweight/obese Czech children
		Jóźków et al. (2011)	C-2745T	The PA was determined with use of the IPAQ	C-2745T polymorphism does not influence the level of physical activity in healthy men
Angiotensin I-converting enzyme	*ACE*	Suchánek et al. (2009)	rs1799752	9 weeks of training, 3 times a week, 60 min per day (65% of maximal heart rate)	Weight loss and decreased in BMI and fat mass in the two genotypes studied in Czech obese woman
		Cięszczyk et al. (2016)		12 weeks of moderate-to-intense aerobic training	The results showed that the training improve (weight, BMI, and body composition) independent of the genotype in Polish women

Continued

Table 21.1 Genes related to exercise-induced weight loss interventions—cont'd

Gene name	Sigla	Author	SNP	Exercise protocol	Conclusion
Peroxisome proliferator-activated receptors	*PPAR (PPARG)*	Zarebska et al. (2014)	rs1801282	12 weeks of aerobic training	PPARG genotype may modulate changes in body mass measurements induced by training
Leptin	*LEP*	Walsh et al. (2012)	rs7799039	Habitual PA 12 weeks of RT program	LEP19 G > A was associated with habitual PA and the body composition response to RT in European-derived American volunteers
β2 adrenergic receptor	*ADRB2*	Masuo et al. (2005)	rs1042714	Low-calorie diet and everyday aerobic exercise	Gly16 allele resistant to weight loss in overweight men
β3 adrenergic receptor	*ADRB3*	Shiwaku et al. (2003)	rs4994	Exercise and a low-calorie diet	Women with the Arg64 allele lost less weight than women without the allele
		Phares et al. (2004)		24 weeks of aerobic exercise training	Arg64 allele showed a great loss of fat mass

BMI, body mass index; *HR*, heart rate; *PA*, physical activity; *RT*, resistance training.

had a moderate level of PA (3 to <6 METs) had the effect of FTO risk allele on BMI attenuated (Celis-Morales et al., 2016).

Mitchell et al. (2010) analyzed another single nucleotide polymorphism (SNP) rs8050136 on FTO gene in postmenopausal women evidenced that AA homozygous women exposed to 6 months moderate or intense physical exercise lost significantly more weight than the C allele carriers. Moreover, Xiang et al. (2016) performed a systematic review and meta-analysis about FTO gene and weight loss in diet and lifestyle interventions. Results showed that individuals with the AA and AT genotype (the risk allele for obesity) had greater weight loss than those with the TT genotype. In conclusion, the authors suggested that individuals carrying the risk allele in homozygosis might lose more weight after dietary interventions. The clinical implications are very important, since adopting a physically active lifestyle and diet can reduce the genetic susceptibility to obesity in the presence of FTO variants.

21.2.2 MC4R gene

The melanocortin 4 receptor (MC4R) is known as the main regulator of food intake and energy expenditure (Hebebrand et al., 2010). The MC4R belongs to the family of seven transmembrane proteins with G receptors. The polymorphisms in the MC4R coding region have been reported to be associated with obesity in humans. In addition, variants outside of the coding region probably influence its expression and have been associated with a predisposition to excess body weight (Leońska-Duniec et al., 2016).

The C/T polymorphism (rs17782313) of MC4R is related to increased BMI and risk of type 2 diabetes. Leońska-Duniec et al. (2017) evaluated weight loss in a group of Polish women before and after a 12-week aerobic training program. Participants with CC and CT genotypes had higher glucose levels throughout the study period compared to TT genotype. However, there was no relationship between C allele and an increase in BMI. In addition, no interaction was observed between MC4R C/T polymorphism and PA.

Another study verified a possible association between the C-2745T polymorphism in the MC4R gene and the level of PA in a men cohort. There was no relationship between the level of PA and the C-2745T polymorphism, showing that C-2745T polymorphism does not influence the level of PA in healthy men (Jóźków et al., 2011). Li et al. (2010) genotyped 12 SNPs at obesity susceptibility loci, including rs17782313 of the MC4R gene in European adults. The authors found that the genetic predisposition to increase BMI and obesity risk is mitigated by a physically active lifestyle. In addition, Zlatohlavek et al. (2013) after genotyped Czech children, evidenced that carriers of CC genotype lost significantly more body weight compared to noncarriers, even when adjusted for sex, age, and baseline values. In conclusion, variants of the MC4R gene may modify the impact of a physical exercise intervention; however, further studies are needed to determine the influence of physical exercise on the polymorphism.

21.2.3 ACE gene

The angiotensin I-converting enzyme (ACE) is the most studied gene in relation to performance in sport (Leońska-Duniec et al., 2016). The ACE insertion/deletion (ACE I/D, rs1799752) polymorphism has been related to improvements in performance in a variety of populations. Notably, I allele is associated with endurance sports and high repetition training program, while D allele is associated with power training (Ma et al., 2013).

The knowledge about the ACE gene in athletic performance has been reported for decades, but there are few articles linking gene polymorphisms with physical exercise interventions for weight loss. Moran et al. (2005) found that insertion/deletion (I/D) polymorphism is related to obesity in Greek adolescents, showing a strong association between the polymorphism and subcutaneous fat in the female sex. The D allele was associated with increased fat thickness.

Suchánek et al. (2009) evaluated obese women (12 II and 12 DD homozygotes) who underwent 9 weeks of training, three times a week, at 65% of maximal heart rate. The intervention provided weight loss, decreased BMI, and reduced fat mass in the two genotypes studied; however, there was an increase in basal metabolic rate only in II genotype.

Cięszczyk et al. (2016) analyzed ACE gene polymorphism in a group of healthy women before and after 12 weeks of moderate-to-intense aerobic training. The results showed that the training improves almost all the parameters (weight, BMI, and body composition) independent of the genotype. In addition, there was a significant increase in maximum expiratory volume (VEmax, L/min) in homozygous genotypes (II and DD), but not for heterozygous ID. In conclusion, further studies are needed to establish ACE gene interactions and PA for weight loss.

21.2.4 PPAR genes

Peroxisome proliferator-activated receptors (PPARs) are transcription factors belonging to the nuclear receptor family responsible for the regulation of various biological processes, in which three isoforms derived from the same gene have been identified and studied more frequently: PPARα, PPARβ, and PPARG (Monsalve et al., 2013). The PPAR genes have an important role in regulating lipid metabolism, adipocyte differentiation and proliferation and insulin sensitivity (Huang et al., 2011). The expression of the PPAR gene is increased in the adipose tissue of obese individuals compared to normal weight ones, being reduced during weight loss (Swarbrick et al., 2001), and it is considered a genetic markers for obesity and has an impact on weight control during exercise (Leońska-Duniec et al., 2016).

Most studies have investigated PPARG Pro12Ala polymorphism, since variant alleles may influence weight loss. Zarebska et al. (2014) analyzed changes in body mass of women after 12 weeks of aerobic training supervised by a physical education teacher. The results suggest that the PPARG genotype may modulate changes in body mass

measurements induced by training. Women who had the homozygous Pro12/Pro12 genotype had a greater decrease in fat mass (kg and percentual) compared to patients with the 12Ala allele. In conclusion, the PPARG 12Ala variant may impair the positive effects induced by training in women.

21.2.5 LEP gene

Leptin is a hormone secreted mainly in adipose tissue that plays an important role in the regulation of energy expenditure and food intake (Lenard and Berthoud, 2008). Leptin is encoded by the LEP gene located on chromosome 7, which is transcribed into a protein of 167 amino acids with a molecular weight of 16 kDa (Yan et al., 2015). The gene has several polymorphisms described, however, the LEP G-2548A polymorphism (rs7799039), located in the promoter region, is the most studied and most common. The association between this polymorphism and obesity is still controversial, and conflicting results have been reported in different populations (Mammes et al., 2000; Boumaiza et al., 2012; Sahin et al., 2013; Ferreira-Julio et al., 2014). It appears that this polymorphism may influence the production and secretion of leptin by adipocytes in obese individuals (Mammes et al., 2000).

The level of PA practiced may also be hereditary, in which case, heritability studies have shown that from 20% to 70% of the variation in PA levels are genetically determined (Stubbe et al., 2006). Variations in the DNA sequence in the LEP gene may induce mild dysfunction in the leptin-mediated signaling pathway and impair its peripheral effects (Lakka et al., 2004; Minokoshi et al., 2002) and thus attenuate the favorable effects of regular PA.

LEP increases the spontaneous practice of PA and this is due to the increase in the cocaine- and amphetamine-regulated transcript (Kristensen et al., 1998). The amount of spontaneous PA practiced has been related to the activity of the sympathetic nervous system, which is also activated by LEP (Tang-Christensen et al., 1999). Thus, differential circulating levels of LEP due to genetic variation may influence the LEP mediated increase of the cocaine-and-amphetamine-regulated transcript and/or stimulation of sympathetic nervous system activity resulting in higher or lower levels of PA (Walsh et al., 2012).

21.2.6 ADRB2 and ADRB3 genes

The family of beta-adrenergic receptors includes beta 2 adrenergic receptor (ADRB2) and the beta 3 adrenergic receptor (ADRB3). These proteins are encoded by the same name genes and are involved in energy homeostasis by the mediation of catecholamine-induced activation of adenylate cyclase through the action of G proteins (Park et al., 2005; Chou et al., 2012).

Genetic polymorphisms in these genes may alter lipolysis and thermogenesis rate, and predisposition to obesity (Chou et al., 2012) and resistance to weight loss. Studies have shown that the polymorphism Gln27Glu (rs1042714) that consist in an amino acid

change in codon 27, may limit ADRB2 downregulation and consequently affects the changes in body weight (Lange et al., 2005; Masuo et al., 2005). After 24-month weight loss program consisting of a low-calorie diet and everyday aerobic exercise, authors evidenced that overweight men with Gly16 allele resistant to weight loss. Also, the higher frequency of this allele was observed in those who regained body weight after successful initial weight loss at 6 months (Masuo et al., 2005).

Furthermore, the polymorphism Trp64Arg (rs4994) in codon 64 of the ADRB3 gene has been associated with fat accumulation and excess of body weight due to a low gene activity (Clement et al., 1995; Widén et al., 1995). Study with women who underwent lifestyle intervention combining exercise and a low-calorie diet showed that those with the Arg64 allele lost less weight than women without the allele, suggesting that the variant allele is associated with difficulty in losing weight (Shiwaku et al., 2003). On the other hand, the authors found and demonstrated an opposite allelic response to exercise. They evidenced that individuals with Arg64 allele showed a great loss of fat mass after 24 weeks of aerobic exercise training compared to noncarriers (Phares et al., 2004). Another evidence suggests that the presence of the Trp64Arg variant of the beta3-adrenergic receptor gene may not work as the impediment of weight reduction (Kuriyama et al., 2008).

These results support the theory that polymorphism in adrenergic receptor genes contributes to interindividual changes in body weight in response to physical exercise (Leońska-Duniec et al., 2018). Thus, despite conflicting results in the literature, ADRB2 and ADRB3 polymorphisms may influence weight loss and, in this way, further studies are necessary to clarify their metabolic functions (Szendrei et al., 2016).

21.3 Epigenetics changes after exercise for weight loss

Epigenetics refers to the study of changes in the patterns of inheritance of gene expression that occur without changes in the DNA sequence. The literature has shown that epigenetics can be considered an important tool for understanding the influence of lifestyle factors on obesity (Bouchard et al., 2010; Moleres et al., 2013; Lavebratt et al., 2012; Lillycrop and Burdge, 2001). The most studied epigenetic mechanisms regarding obesity are DNA methylation in cytosines followed by guanines (CpG regions) and changes in the organization of chromatin by histone modifications (Marti and Ordovas, 2011).

These epigenetic modifications act on the rearrangement of the conformation of DNA or nucleosomes within the nucleus of the cell, which, when they occur, influence, or impede gene transcription through an interaction of several molecular pathways that are still incompletely understood (Bannister and Kouzarides, 2011). Numerous epigenetic modifications are described, however, only DNA methylation and histone acetylation were further investigated in the context of physical exercise (Kouzarides, 2007; Denham et al., 2014) for weight loss.

The epigenome may represent a mechanistic link between genetic variants and environmental factors in determining the risk for obesity and could help explain the absence of heredity in some individuals (Van Dijk et al., 2015a). With advances in high-throughput technologies, the development of wide-ranging epigenome-wide association studies (EWAS) and integration of different sources of genomic information to explore the complex interactions between genotype, epigenome, transcriptome, and environment (Teh et al., 2014; Olsson et al., 2014; Grundberg et al., 2013; Rönn et al., 2015). The results obtained so far are promising in an attempt to explain the variation in susceptibility to obesity.

Obesity also originates during embryonic development, thus, individuals who were exposed to a given nutrient supply prior to birth or in early childhood may present an increased risk of obesity and metabolic disease in adult life (McMillen et al., 2008; van Dijk et al., 2015b; Bouret et al., 2015). Early epigenetic changes, especially those experienced early in pregnancy, could induce modifications in the major metabolic tissues of offspring that would persist after birth resulting in permanent changes in gene function (McMillen et al., 2008; Bouret et al., 2015). Thus, physical exercise can also be another environmental stimulus that modulates epigenetic modifications and gene expression, not only of the individual performing the physical exercise but also of their progenies.

Physical exercise is an affordable lifestyle option that provides health benefits conferred by regular aerobic and endurance training. There is a reduction in the risk and severity of some cardiovascular, metabolic, and pulmonary diseases, as well as obesity and certain types of cancer (Warburton et al., 2006; Jenkins et al., 2012). In addition, lifestyle and environmental factors can significantly influence gene expression, which is partially modulated by epigenetic modifications (Denham et al., 2014).

The literature has shown the role of physical exercise in altering the expression of various genes in human skeletal muscle tissue. Physical training induces various adaptations at the metabolic level within muscle cells to complement the increased energy needs, and these adaptations require the expression of specific genes, especially when the goal is weight loss (Pareja-Galeano et al., 2014). The availability of other metabolites produced during exercise may affect the activity of epigenetic machinery in specific genes involved in mitochondrial biogenesis. However, the mechanisms by which the regulation of gene expression of important genes involved in metabolic adaptation or metabolic stress is still incompletely elucidated. Analysis of epigenetic changes can be a useful tool to monitor physical training interventions (Pareja-Galeano et al., 2014).

Physical exercise induces the energetic needs, which are met by increased mitochondrial biogenesis via PPAR-coactivator 1 alpha (PGC-1a), nuclear respiratory factor 1 (NRF1), and mitochondrial transcription factor A (TFAM). Increases in the number, size, and activity of mitochondria cause a subsequent elevation in the pool of metabolites of the tricarboxylic acid (TCA) and β-hydroxybutyrate cycle. In addition, this increased oxygen uptake capacity induces a decrease in the intracellular oxygen that blocks 2-oxoglutarate (II) ferrous iron (2-OG-Fe II) dependent enzymes, which in turn block prolyl proteins

hydroxylases (PHDs), jumonji C (jmjC), and ten-eleven-translocation (TET). All these pathways lead to the complex regulation of various epigenetic pathways through the histone demethylases, acetyltransferases, and deacetylases (Pareja-Galeano et al., 2014).

β-Hydroxybutyrate is one of the metabolites that can exert effects on epigenetic regulation, being produced at millimolar levels after prolonged exercise (Shimazu et al., 2013) and a derivative of acetoacetate, which is catabolized by β-hydroxybutyrate dehydrogenase in an NAD^+ dependent reaction. Chemically, β-hydroxybutyrate is similar to butyrate, an inhibitor of class I and II HDACs. All these mechanisms suggest that exercise, which followed weight loss, modulate epigenetic events, due to the fact that the metabolites generated by continuous, acute, moderate, or strenuous exercise control the activity of some highly relevant epigenetic enzymes that regulate gene expression.

Relevant intervention studies in PA have observed differences in the DNA methylation pattern. However, they do not present consistency in the type, duration of the exercise program, characteristics of the study population, and type of tissue examined (Archer, 2015). Interventions for weight loss, including caloric restriction programs or gastric bypass, also observed changes in DNA methylation, however, the results are still inconsistent (Campion et al., 2009; Milagro et al., 2011; Benton et al., 2015; Nicoletti et al., 2016). Although the association between PA and health is well established, research investigating molecular pathways, particularly epigenetic processes, underlying the response to PA remains limited. The characterization of these molecular changes will contribute to the understanding of the biological processes between PA and health outcomes (McEwen et al., 2018). Fig. 21.1 shows the flow chart related to the interaction process between PA, weight loss, and epigenetics.

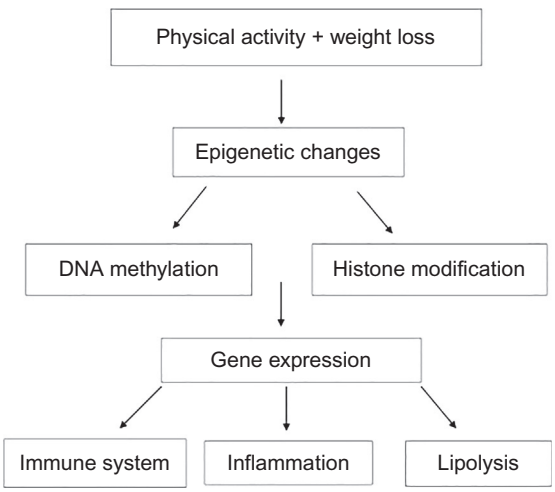

Fig. 21.1 Process of interaction between physical activity, weight loss, and epigenetic which can modulate gene expression variation acting on the molecular pathways of the immune system, inflammation, lipolysis, among others.

21.4 Metagenomics: How weight loss by exercise training influences gut microbiota

The human gut microbiota includes approximately 100 trillion microbes and Firmicutes (60%–65%), Bacteroidetes (20%–25%), and Proteobacteria (5%–10%) genus is the majority of human gut microbiota. However, it is important to note that there is considerable interindividual variability in microbial diversity and composition (Rosenbaum et al., 2015). Moreover, according to specific conditions of each gut portion (e.g., pH, antimicrobial peptides, and amount of oxygen), bacterial density varies along the gastrointestinal tract (Donaldson et al., 2016).

The commensal human gut microbiota has been associated with health and risk of disease and, consequently, changing its composition is linked with changes in human behavior (Sekirov et al., 2010; Shanahan, 2012). Obesity-related disorders have been linked with alterations in the microbiota (Qin et al., 2012). Of note, studies showed that both the diversity of the microbiota and the Bacteroidetes/Firmicutes ratio are decreased in obese individuals. Thus, obese microbiota can favor fat deposition and consequent weight gain (Compare et al., 2016).

Many studies provide evidence that the manipulation of gut microenvironment could be useful to prevent obesity or contribute to weight reduction (Compare et al., 2016). In the line of this, evidence suggests that exercise may also modify human microbiota (Queipo-Ortuno et al., 2013). Exercise and dietary factors has been shown to be an important factor in the relationship between microbiota diversity, host immunity, and host metabolism (Clarke et al., 2014). Also, it has been more evident that gut microbiome is an essential supplier of an extensive range of metabolites that influence mitochondrial function and biogenesis within the skeletal muscle to stabilize host metabolism (Franco-Obregón and Gilbert, 2017). Table 21.1 shows exercise-induced weight loss interventions and gut microbiota studies.

In animal models, it is well described that exercise-training changes significantly gut microbiome. In diet-induced obesity mouse, PA was shown to modulate gut microbiota (Evans et al., 2014). Also, exercise training improves bacterial diversity in obese and hypertensive rats by increasing the amount of *Firmicutes* and decreasing the content of *Proteobacteria* (Petriz et al., 2014). The same authors showed that *Lactobacillus* genus presented higher abundance after exercise obese rats; hypothesizing that exercise may have some influence on gastrointestinal acidity through the production of acidic compounds (Petriz et al., 2014).

The effects of exercise training on the microbiota of diet-induced obesity rats were gut segment dependent and more extensive in the distal gut. The high-intensity interval training (HIIT) increased microbiota diversity and Bacteroidetes/Firmicutes ratio. The authors concluded that exercise training is able to oppose some of the obesity-related changes in gut microbiota, including lower metagenomic indexes of metabolism (Denou et al., 2016).

Another study supports the idea that prevention of weight gain by exercise is associated with a relative increase in Bacteroidetes and increase of intestinal microbiota diversity (Evans et al., 2014). Exercise also reduced the percentage of the family *Lactobacillaceae* (Evans et al., 2014). It is important to note that *Lactobacillaceae* has a crucial role in sedentary lifestyle-associated weight gain; and, depending on the Lactobacillus species and experimental model, the effects of Lactobacillus on weight gain and obesity can be quite different (Bervoets et al., 2013). Moreover, exercise training was associated with a reduction of *Erysipelotrichaceae* and *Turicibacteraceae* levels, which are families of bacteria associated with obesity, bile acid regulation, and gut inflammation (Evans et al., 2014). Notably, changes in the microbiota by exercise training have also been associated with improvements in metabolic parameters (Queipo-Ortuno et al., 2013), however, the exact mechanisms for how exercise induces changes in the microbiota are still unclear but could involve cross-feeding of metabolites (Cook et al., 2016).

In humans, a major study conducted on elite players indicated that both diet and exercise determined the microbial biodiversity of the gut (Clarke et al., 2014). However, is scarce in the literature studies that evaluate the modifications in the microbiota after exercise aiming weight loss (Table 21.2).

According to microbiota diversity, athletes and low BMI individuals had significantly higher proportions of the genus *Akkermansia* levels than the high BMI group. *Akkermansia muciniphila* abundance has been shown to have an inverse correlation with obesity and metabolic disorders in mice and humans (Karlsson et al., 2012; Everard et al., 2013). Indeed, interventions with caloric restriction and exercise in obese adolescents have demonstrated changes in microbial composition. Good (>4 kg of weight loss) and bad (<2 kg of weight loss) responders showed different baseline microbial composition and changes after intervention were associated with weight loss (Santacruz et al., 2009).

The main studies evaluating physical exercise, weight loss, and gut microbiota are demonstrated in Table 21.3.

21.4.1 Possible mechanisms connecting exercise-induced weight loss interventions and gut microbiota

The beneficial effects of exercise on gut microbiota and its consequent variations in health may be related to changes to two points: short-chain fatty acids (SCFA) profile and bile acids secretion (Matsumoto et al., 2008).

Interestingly, diet compounds are able to influence the diversity, composition and metabolic activity of gut microbiota, as well as its fermentation capacity and SCFA production (Liu et al., 2014; O'Sullivan et al., 2015). For instance, carbohydrate fermentation is considered beneficial for the host through specific SCFA production, while protein fermentation gives rise to a wide variety of compounds, which some could have negative effects for gut health when present at an excessive concentration (Andriamihaja et al., 2015).

Table 21.2 Exercise-induced weight loss interventions and epigenetic studies.

Author	Population studied	Exercise protocol	Results	Conclusion
McEwen et al. (2018)	Healthy postmenopausal women (55–70 years of age) and control group	6 months of nine sessions of 2 h focused on reducing sedentary behavior	Alterations at epigenetic modifications that correlated with change in percent body weight over a 6-month period at 12 genomic loci	Potential biological link between body weight changes and epigenetic processes
Moleres et al. (2013)	Overweight or obese adolescents	10 weeks intensive program period of nutritional and physical advice	Five regions (in or near *AQP9*, *DUSP22*, *HIPK3*, *TNNT1*, and *TNNI3* genes) showed differential methylation levels between high and low responders to the program	An epigenetic score could be used to predict body weight changes
Wu et al. (2015)	Obese and lean children	Sedentary behavior and physical activity were investigated	Methylation levels at seven CpG sites of the FAIM2 promoter were significantly associated with sedentary behavior. Differences between the methylation levels at four CpG sites in obese and lean with high or moderate physical activity level < 150 min/week	There are differences in FAIM2 promoter methylation with sedentary behavior and physical activity
Rönn et al. (2013)	Healthy men	One session of 1-h spinning and two sessions of 1-h aerobics. About 1.8 sessions/week	Global DNA methylation changed and 17,975 individual CpG sites in 7663 unique genes showed altered levels of DNA methylation after the exercise intervention	There is differential DNA methylation in genes involved with obesity
Murashov et al. (2016)	C57B/6J mice	Wheel-running by 7.1 ± 0.3 km/d over a 12 week period	Prolonged exercise affected gene methylation patterns and micro-RNA content in the sperm of fathers	Paternal exercise produces offspring with an economic phenotype, potentially via miRNA-induced modification of sperm
Bajpeyi et al. (2017)	Healthy males	A cycle ergometer at 50% maximal oxygen consumption until reaching an energy expenditure of 650 kcal	When exercise: the −1 nucleosome is repositioned away from the regulatory −260 nucleotide methylation site in high responders, those exhibiting a significant decrease in −260 nt methylation. In high responders there is a significant decrease in intramyocellular lipid content after exercise	A potential target for epigenetic modification of the *PGC1α* promoter to stimulate the therapeutic effects of endurance exercise in skeletal muscle

Table 21.3 Exercise-induced weight loss interventions and gut microbiota studies.

Author	Population studied	Exercise protocol	Results	Conclusion
Petriz et al. (2014)	Obese rats nonobese Wistar rats and spontaneously hypertensive rats	Running at 12.5 m min^{-1}, 30 min per day, 5 days per week for 4 weeks	*Lactobacillus* were shown to be enriched after exercise (obese rats)	Exercise training alters gut microbiota from an obese and hypertensive genotype background
Denou et al. (2016)	Male rats feeding with a high-fat diet	Running at 8 m min^{-1} on a 5% grade and treadmill speed was gradually increased	Exercise training increased the alpha diversity within the phylum Bacteroidetes in both the cecum and colon and increased the Bacteroidetes/ Firmicutes ratio in the cecum	Exercise training directly opposed some of the obesity-related changes in gut microbiota
Evans et al. (2014)	Male C57BL/6 littermates (5 weeks)		Exercise induced changes in the percentage of major bacterial phyla	Exercise induces a unique shift in the gut microbiota
Nadal et al. (2009)	Obese adolescents	Caloric restriction and exercise intervention for 10 weeks	High responders (weigh loss >4 kg) showed increase of Bacteroides/ Prevotella	
Santacruz et al. (2009)	Overweight/ obese adolescents	Caloric restriction and exercise intervention for 10 weeks	Greater change in bacterial group abundance for high responders (weigh loss >4 kg)	

Indeed, high-fat diet is related to Gram-negative bacteria growth, which would be damaging for gut health (Liu et al., 2014). Despite several studies regarding the effects of diet, exercise has been revealed as another factor capable of influencing SCFA production, specifically n-butyrate.

Physical exercise by change butyrate production, decreased intestinal luminal pH and turned the gut more favorable for the proliferation of some bacterial species In addition, butyrate can promote cell differentiation and cell cycle arrest, inhibit the enzyme histone deacetylase, and decreases the transformation of primary to secondary bile acids (Wong et al., 2006) (Fig. 21.2).

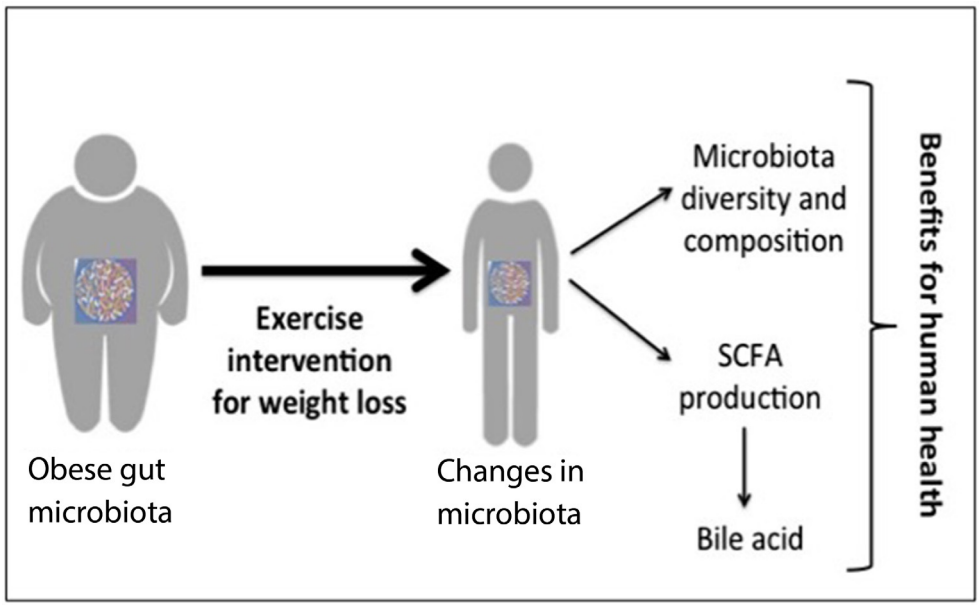

Fig. 21.2 Gut microbiota and exercise intervention for weight loss.

21.5 Conclusion and futures perspectives

In the first instance, personalized weight loss interventions refer mainly to the genetic background in which gene polymorphisms are associated with the individual's response. However, more and more studies have been showing the role of epigenome and microbiome in the modulation of gene expression on body weight management. In line of this, genetic and epigenetic markers are been evidenced and these could predict the outcome of exercise-induced weight loss interventions. The integration of genomics, nutrigenetic, epigenetic, and metagenomic data in fitness and sport may help health professionals to adopt personalized strategies and thus optimize the individual's response to interventions and achieve better results. In this way, the field of exercise genomics to be ready for substantial progress in the next decade, mainly due to the development and application of high-performance technologies.

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CHAPTER TWENTY TWO

Beneficial effect of physical exercise on telomere length and aging, and genetics of aging-associated noncommunicable diseases

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22.1 Introduction

Aging has been intensely studied by scientists of all times. Although many theories have been proposed to explain the causes and processes involved in aging, the approach that aging takes place in four levels has lately gained attention. The theory supports that each level represents a biological scale, and alterations of the functions in each level lead to the phenotype of aging and age-related diseases (Zhang et al., 2015). These four layers would be: (1) general physical deterioration of the organism; (2) dysfunction of systems involved in regulation of body physiology, that is, the immune, metabolic, and endocrine systems; (3) altered cellular function, including increases in the number of senescent cells and in the production of reactive oxygen species (ROS), loss in the ability of endoplasmic reticulum response to misfolded proteins and reduction in the ability to degrade specific molecules using the ubiquitin-proteasome and autophagolysosome systems; and (4) failure to maintain biomolecules (Zhang et al., 2015). At cellular level, six factors have been considered responsible for the process of cellular senescence, namely (1) telomere shortening, (2) DNA double-strand breaks and DNA damage response, (3) epigenetic alterations, (4) aneuploidy, (5) oxidative stress and mitochondrial dysfunction, and (6) inflammation, energy sensing, and altered metabolic regulation (Bhatia-Dey et al., 2016). In this chapter, we will focus on the mechanisms associated with telomere shortening in aging and we will describe the potentially beneficial effects of physical activity.

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22.2 Telomere length (TL) and aging

In the middle of the last century, Muller (1938) and McClintock (1941) defined telomeres as functional ends of chromosomes, unlike random broken endings. A couple of decades later, Hayflick and Moorhead (1961) and Hayflick (1965) described what has been named "Hayflick limit." Their works illustrate the diploid karyotype maintenance in human fibroblasts strains, in serial cultivation for long periods of time, and characterize cellular degenerations that appear in what they called Phase III (terminal period during which the intervals of time between each cell division are greater) as loss of growth potential (Hayflick and Moorhead, 1961). Thus, they established that in vitro cultures of human diploid cell strains had a finite life time, which seemed to be more related to the number of divisions than to the subcultures split ratio (Hayflick, 1965). Olovnikov, in 1971, proposed that during DNA replication, in the division of somatic cells, a part of telomeric DNA is lost due to the end-underreplication, so that cells would already have a determined number of divisions and, after a critical loss of telomeric DNA, cells would change from a young to an old phenotype (Olovnikov, 1971). After 20 years, Harley et al. (1990) confirmed this hypothesis in human fibroblast and gave it the name of the mitotic clock or telomere hypothesis. Allsopp's work (1992) concluded that TL could predict the replicative capacity of these cells. In addition, previously in 1971, Olovnikov had proposed that, unlike somatic cells, germ cells and malignant cells could express a type of DNA polymerase that protects against telomere shortening (Olovnikov, 1971). This polymerase was identified as telomerase in the late 1980s by Greider and Blackburn (1985, 1989) and Morin (1989), among others.

In a cohort study, Tedone et al. (2014) compared the prevalence of age-related diseases and TL across five groups of subjects: (i) semisupercentenarians (105–109 years), (ii) centenarians (100–104 years), (iii) centenarians' offspring, (iv) age- and gender-matched offspring of parents who both died at an age in line with life expectancy, and (v) age- and gender-matched offspring of both nonlong-lived parents. Results indicated that both centenarians and their offspring may be characterized by a better TL maintenance, contributing to their longevity and healthy aging. The same study also showed that semisupercentenarians have considerable shorter telomeres compared to centenarians, suggesting a progressive impairment of TL maintenance mechanisms over the transition from centenarian to semisupercentenarian age (Tedone et al., 2014).

22.2.1 Mechanisms associated with telomere shortening in aging

Telomere maintenance is influenced by many genetic, environmental, and lifestyle factors (Fig. 22.1). Lin et al. (2012) extensively reviewed the effects of various factors including stress-related psychiatric conditions (posttraumatic stress disorder, major depressive disorder and bipolar depressive disorder with and without anxiety) on TL. These factors

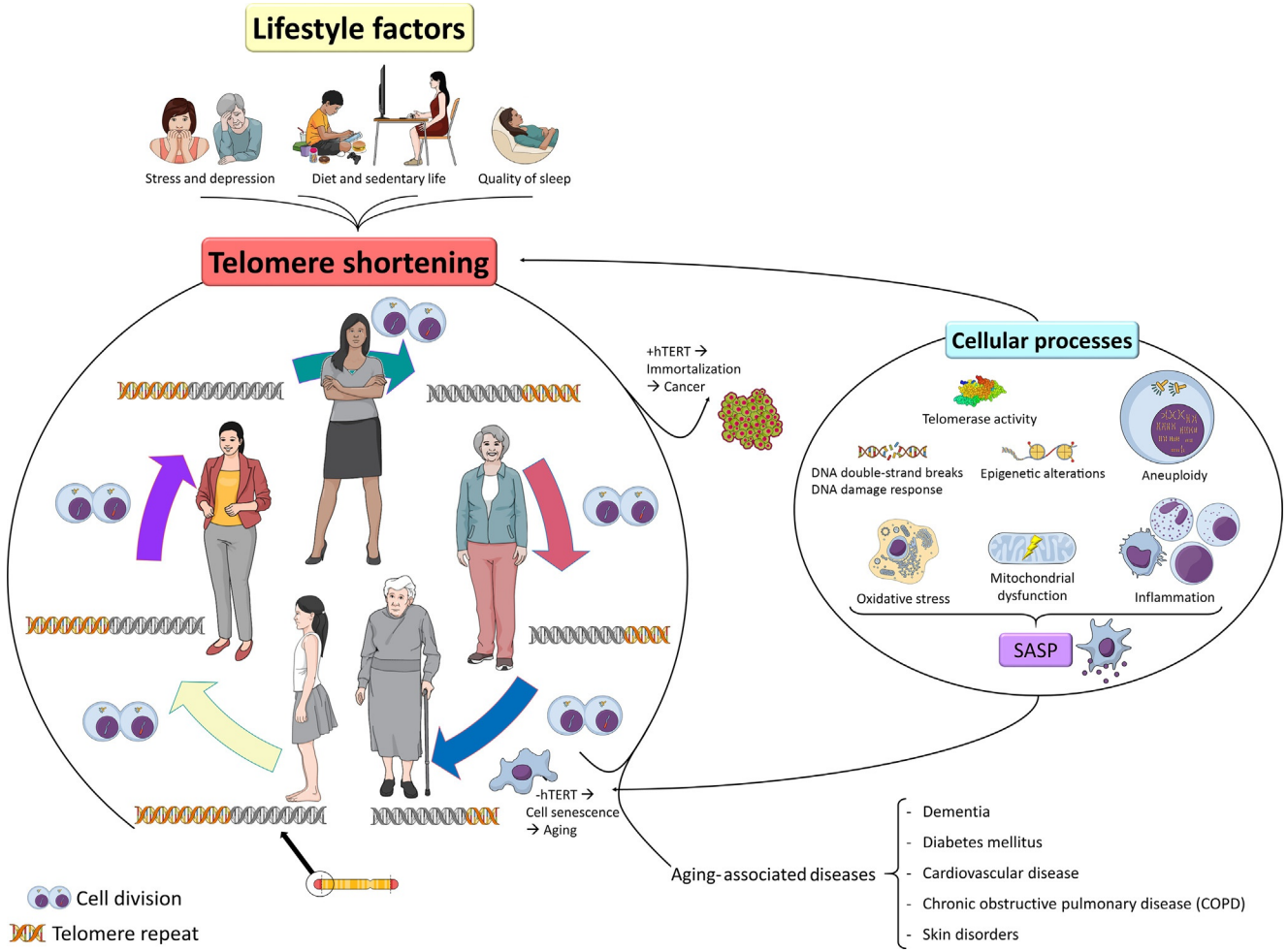

Fig. 22.1 Schematic representation of the interactions between different life factors, alterations in cellular processes and telomere shortening in the course of aging, as well as their relationship with aging-associated diseases.

could affect TL in such a way that, in the same cell type, longer telomeres could be found in the elderly than in young subjects (Blackburn, 2000).

Recently, different mechanisms associated with age-related telomere shortening have been proposed, including stress hormones, inflammation, and oxidative stress (Lin et al., 2012; Arsenis et al., 2017). However, telomerase activity has received more attention for its direct role in DNA replication (Boccardi and Paolisso, 2014). Thus, in a recent longitudinal study evaluating TL in peripheral blood mononuclear cells (PBMCs) in 216 participants from 20 to 90 years, Lin et al. (2015) described that age-related changes in TL occur at different speeds in different individuals and cell types. Thus, telomere shortening would be influenced by telomerase activity, the percentage of naive T cells and changes in physiological conditions, such as glucose or interleukin (IL) concentrations.

22.2.1.1 Telomerase activity

Telomerase activity represents a key factor in telomere maintenance given its capacity to regulate changes in telomere shortening rate and telomere stability. Telomerase is a reverse transcriptase enzyme formed by a catalytic subunit (TERT) and an RNA template (TERC). Specifically, cells with high proliferation capacity (e.g., primary germline cells, lymphocytes, smooth muscle cells, and fibroblasts) and cells during fetal development exhibit high telomerase activity (Boccardi and Paolisso, 2014). However, in most somatic tissues there is a repression of its activity, probably to prevent cancer onset in long-lived mammals (Shay, 2018). In this line, an experimental work that suppressed telomerase activity observed telomere shortening and subsequent senescent phenotype (Yu et al., 1990). Conversely, in experiments in which its activity was promoted, somatic cells exhibited telomere elongation and were shown to be immortal (Bodnar et al., 1998; Counter et al., 1998).

A study carried out by Iwama et al. (1998) explored the relationship between telomerase activity, TL and age in PBMCs of 124 healthy individuals, whose ages ranged between 4 and 95 years. The study concluded that terminal restriction fragments (TRF) length, a representation of TL, decreased with age at 84 base pairs (bp)/year, in individuals from 4 to 39 years, and 41 bp/year, in individuals over 40 years of age. Regarding telomerase activity, Iwama et al. (1998) also described that up to 39-year-old age, there was a decrease in telomerase activity; however, in subjects over 40 years, two groups were differentiated, one in which enzyme activity was very low, but stable, and another in which no activity was detected.

In a cross-sectional and longitudinal study, Soerensen et al. (2012) demonstrated that variations in the genes encoding TERC subunit are related to both leukocyte TL (LTL) and longevity in humans. Later, Lin et al. (2015) described an age-associated decrease in telomerase activity in both resting T and B cells and in stimulated T cells in vitro.

Finally, it is convenient to highlight that, in certain situations, telomerase activity would cause a telomere catastrophe. Thus, in a recent study, Margalef et al. (2018) have described that telomere replication is inhibited by inappropriate telomerase binding to reversed replication forks driving telomere shortening.

22.2.1.2 Oxidative stress

Different processes, including oxidative metabolism products of cellular respiration in mitochondria, produce highly reactive ROS, which are able to induce damage on cellular components (proteins, lipids, and DNA) and form molecules that can alter different metabolic pathways. In normal conditions, the effect of these ROS is counteracted by various antioxidant systems; however, an excessive accumulation of ROS in cells, tissues or organs can cause oxidative stress, leading to DNA damage (Arsenis et al., 2017; Chilton et al., 2017). In addition, mitochondria with DNA damage produce a greater amount of free radicals, which in turn cause greater deterioration of mitochondrial DNA, thus forming a vicious circle.

In recent decades, one of the most accepted theories of aging is the free radicals or oxidative stress theory. One consistent line of evidence supports that different oxidative-stress genes are linked with both telomere shortening and aging (Starr et al., 2008). Actually, several antioxidant molecules such as glutathione, vitamins, and antioxidant enzymes are associated with longer telomeres, and their content and/or their function decrease with age. In this context, telomeric G triplet is particularly susceptible to oxidative stress (Hewitt et al., 2012). Moreover, the reparation of damaged telomeric DNA is less efficient when compared to coding regions of the genome, leading to shorter telomeres and cumulative and irreparable damage (Fumagalli et al., 2012).

Paradoxically, there is a delicate balance between TL and oxidative stress. While severe oxidative damage can be harmful to telomeres, mild exposure to oxidative stress can play a protective role over TL by enhancing oxidative damage repair systems (Radák et al., 2003), as observed in sperm telomeres (Mishra et al., 2016). In fact, under oxidative conditions, telomerase translocates to the mitochondria where it appears to play a protective role against stress (Haendeler et al., 2009). Additionally, a certain amount of ROS may also be protective by increasing antioxidant defense systems (Chilton et al., 2017). In this sense, overexpression of the antioxidant enzyme superoxide dismutase reduces telomere shortening in human fibroblasts (von Zglinicki et al., 2000). In conclusion, it appears that, as long as ROS concentration is maintained within a determined threshold, ROS can aid in telomere maintenance.

22.2.1.3 Inflammation

Aging has been associated with immunological alterations in the adaptive immune system, a process known as *immunosenescence*. These changes promote a systemic low-grade pro-inflammatory phenotype known as *inflammaging*. This process is characterized by

(1) an increased expression of immune responses- and inflammation-related genes, (2) an activation of the nuclear factor kappa B (NF-κB) signaling pathway, and (3) greater concentrations of pro-inflammatory cytokines in serum, for example, IL-6, IL-1α, IL-1α receptor antagonist (IL-1Rα), IL-18, as well as pro-inflammatory mediator tumor necrosis factor (TNF)-α (Salminen et al., 2012a,b; Wu et al., 2015; Youm et al., 2013; Zhang et al., 2016). Senescent cells also exhibit modifications in gene expression of cell-cycle inhibitors or activators and an increase in the secretion of different markers, including pro-inflammatory factors such as several cytokines or growth factors among others, that change the tissue microenvironment, process known as senescence-associated secretory phenotype (SASP) (Zhang et al., 2016).

In a cross-sectional study completed in 798 men and women (aged 55–79 years), Shin and Baik (2016) found that increased serum homocysteine levels and elevated inflammation, measured as serum high-sensitive C-reactive protein (hs-CRP), were associated with reduced LTL. Similar results were also observed by Arai et al. (2015) in a longitudinal study evaluating centenarians and semisupercentenarians and their direct (offspring) and unrelated (spouse of offspring) family, as well as community-living octo- and nonagenarians (85–99 years), covering a wide range of chronological ages from around 50 up to 115 years. Authors identified that populations between 85 and 104 years of age, and the offspring of centenarians, maintained long telomeres and low levels of basal inflammation.

After a comprehensive review on the relationship of telomere shortening and inflammation in aging, Zhang et al. (2016) reported that an increase in pro-inflammatory factors, such as TNF-α, IL-6, CRP, and interferon (IFN)-γ, mediates this interaction. In addition, this review highlights how a model of senescence-derived telomere shortening, where deficient mice in telomerase subunits ($TERC^{-/-}$ and $TERT^{-/-}$) were generated, ended up presenting chronic inflammation, probably due to cytokine and chemokine release into the environment by senescent cells.

Opposing data have been also observed. Indeed, mutations in human TERT (hTERT) and human TERC (hTERC) have been shown to increase pro-inflammatory cytokine levels, causing alveolar stem cells senescence (Chen et al., 2015). In a similar study, Kang et al. (2018) discovered that $TERC^{-/-}$ aged-mice showed a large lung inflammation and that telomere dysfunction led to NLRP3 inflammasome activation in macrophages.

22.2.2 TL and aging-associated diseases

As previously mentioned, there are clear evidence linking the accelerated telomere shortening and aging, with an estimated TL decrease rate between 24.8 and 27.7 bp/year. On the contrary, TL is negatively associated with numerous chronic conditions and age-related complications, including cancer, coronary heart disease, heart failure,

diabetes, obesity, chronic obstructive pulmonary disease (COPD), osteoporosis, or skin disorders, among others. In the next section, the relationship between telomere shortening and some of the most prevalent diseases in the elderly population will be briefly discussed (Fig. 22.1).

22.2.2.1 Cancer

The length and integrity of telomeres play a key role in the development of cancer (Artandi and DePinho, 2010). Several studies suggest that a long LTL is associated with an increased risk to develop several types of cancer, possibly due to a greater replicative capacity and the accumulation of abnormalities associated with longer telomeres (Chilton et al., 2017). However, most research supports that shortened telomeres can have oncogenic effects by fusing with other uncapped telomeres and generating destabilizing genomes (Artandi and DePinho, 2010). One possible explanation for these discrepancies is that telomere uncapping has an effect on anticancer in young people, but that the shortened, uncapped, and depleted telomeres of the elderly are potentially oncogenic in this population group (Yang et al., 2016).

Progressive telomere shortening can induce genomic instability and activation of the response to DNA damage (Oeseburg et al., 2010). Under normal conditions, this will result in the activation of a series of associated factors that ultimately include the phosphorylation of the tumor suppressor protein, p53. If the p53 pathway functions correctly, senescence or apoptosis will begin and tumorigenesis will be inhibited. But when the p53 pathway is altered, inhibition of tumorigenesis does not occur in the presence of telomeric dysfunction (Deng et al., 2008). Another remarkable aspect is that 85%–90% of malignant tumors are positive for telomerase. This finding indicates that telomerase activity is a key component in the malignant transformation of cells (Shay and Bacchetti, 1997). In fact, when cancer cells and healthy cells are compared, higher telomerase activity and shorter TL have been detected in most cancer cells (Shammas, 2011).

Clinical data reveal that a decrease in LTL is associated with an increase in the overall incidence of cancer and mortality (Arsenis et al., 2017). Thus, patients with different types of cancer (e.g., head and neck cancers, breast, ovary, bladder, prostate, stomach, colorectal, lung, or kidney) exhibit shorter telomeres (Oeseburg et al., 2010). In addition, several studies indicate that people with shorter telomeres, as observed in the elderly, seem to have an increased risk of developing cancer (Wu et al., 2003). Also, individuals with a congenital deficiency in the telomerase RNA gene or with genetic disorders that lead to shorter telomeres, have a higher risk of developing premature aging, predisposition to cancer, premature coronary disease, vulnerability to infections, progressive failure of the bone marrow, and premature death in adults (Shammas, 2011).

22.2.2.2 Cardiovascular diseases

The presence of abnormally short telomeres has also been related to diverse cardiovascular diseases, including chronic heart failure, late atherosclerosis, ventricular dysfunction, and stenosis of the aortic valve (De Meyer et al., 2011). Yet, some studies have shown contradictory data indicating that there is no significant association between TL and, for example, stroke (D'Mello et al., 2015) or early atherosclerosis (De Meyer et al., 2009). However, despite these discrepancies, most of the scientific community supports the theory that telomere shortening is positively associated with an increased risk of cardiovascular accident, and that this decrease in TL is a consequence of the accelerated replacement of leukocytes due to oxidative stress and inflammation (Chilton et al., 2017), a situation that is aggravated in elderly subjects.

There are also studies that argue that a shorter TL is the cause of cardiovascular aging. This theory is based on the heritability of TL and the findings showing that children of parents with cardiovascular diseases have shorter telomeres (van der Harst et al., 2008). Yet again, opposite results indicate that shorter hereditary telomeres do not seem to predispose to atherosclerosis (De Meyer et al., 2009; Willeit et al., 2010). However, not only genetic inheritance but a large number of risk factors (e.g., smoking, sedentary lifestyle, hypertension, obesity, and hypercholesterolemia) could be related to telomeric shortening (Chilton et al., 2017), although the role of some of these factors on LTL has also been challenged in persons suffering from cardiovascular diseases (Neuner et al., 2015).

In recent years it has become apparent that telomerase can also be activated in the cells of the cardiovascular system, including cardiac myocytes, endothelial cells, smooth muscle cells, and fibroblasts (Zurek et al., 2016). Previously, a study evaluating TL in dogs with progressive deterioration of cardiac output and dilated cardiomyopathy identified an increased telomerase expression of 2.4–3.1-folds in the decompensated heart, preserving TL in myocytes. On the other hand, Ki67, a marker of the magnitude of cyclic myocytes, was increased fivefold at the beginning of moderate ventricular dysfunction, 12-fold in the moderate, and 17-fold in the manifest failure (Leri et al., 2001). Telomere shortening in second and fifth generation TERC$^{-/-}$ mice was related with a decrease of cardiac myocyte proliferation, increased apoptosis and cardiac myocyte hypertrophy, resulting in decompensated hypertrophy and heart failure (Leri et al., 2003). Similarly, both in cultured cardiomyocytes and in the mouse myocardium, downregulation of TRF2 caused telomere shortening and apoptosis, so that they could be responsible for chronic heart failure (Oh et al., 2003).

Finally, some authors have proposed TL as a possible biomarker for heart disease (Cawthon et al., 2003; Brouilette et al., 2008). However, patients treated with statins could have greater protection against telomere attrition through telomere repeat-binding factor (TRF) 2. This protective effect may be possibly associated with the antiinflammatory properties observed in statins (Spyridopoulos et al., 2004). A similar protelomeric effect has been attributed to other drugs such as aspirin, angiotensin-converting

enzyme inhibitors, and androgen therapy. The antioxidant capacity of aspirin and its efficacy preventing senescence of endothelial cells seems to inhibit telomerase activation, while angiotensin-converting enzyme inhibitors exert their effect through positive regulation of TERT mRNA in endothelial cells (Chilton et al., 2017).

22.2.2.3 Metabolic disorders

A cardiometabolic disease highly prevalent in old age and an important predictive value of cardiovascular disease is type-2 diabetes *mellitus* (T2D). The biological process of aging and senescence of tissues could be the link between both pathologies (D'Mello et al., 2015). In addition, the cardiovascular problems of diabetic patients often present reinforce this theory. Several studies indicate that the reduction of TL correlates positively with diabetes and its complications (retinopathy, incipient nephropathy, and cardiovascular disease), with progressively shorter LTL in those individuals with more diabetic complications (Arsenis et al., 2017). Even a subclinical presence of insulin resistance has also been associated with telomere attrition (Demissie et al., 2006).

An explanation for telomere shortening in diabetic patients would be an increase in oxidative damage, a condition that, as previously indicated, is exacerbated with age. In addition, other authors have postulated that ROS-induced telomere attrition in diabetic patients has a possible genetic cause. In fact, the investigations of Salpea et al. (2010) showed that patients with T2D who had the UCP2-866A functional variant allele of uncoupling protein 2 (UCP2), had shorter telomeres than their homozygotes for the G allele. The UCP2 protein plays a key role in the electron transport chain acting as a negative regulator of the overproduction of ROS by the mitochondria. This study reflects a clear association between TL in diabetic patients, oxidative stress, and genetic polymorphisms of UCP2.

Unlike the case of cardiovascular diseases, it is unknown whether an excessive telomere shortening is the cause or the consequence of diabetes. Similarly, it has not been possible yet to determine whether TL has a predictive value for the risk of developing T2D since the two large prospective studies that have been carried out have obtained contradictory conclusions (You et al., 2012; Zhao et al., 2014).

In relation to obesity, Müezzinler et al. (2014) conducted a systematic review in order to deepen into the relationship between this metabolic condition and TL. The results of the study showed a tendency to a negative correlation between the body mass index (BMI) and TL (Müezzinler et al., 2014). In the same line, the MONICA Northern Sweden Study demonstrated an association between shorter TL and subjects with self-perceived early aging, who had significantly higher BMI and wider waist circumference than controls (Nordfjäll et al., 2008). However, the high variability between the studies and the weak or moderate statistical significance did not allow obtaining definitive conclusions.

The increase in oxidative stress and the inflammatory processes that accompany obesity, mainly central obesity, are among the key factors linking telomere shortening and obesity (Mundstock et al., 2015). Several studies have shown that BMI and waist circumference (indicators of obesity) correlated significantly with high concentrations of ROS, as well as with biomarkers of DNA damage (Furukawa et al., 2004; Song et al., 2010; Shammas, 2011). Similar findings have also been found in animal models (Furukawa et al., 2004). In addition, in obese mice, a lower expression of the antioxidant activities catalase and superoxide dismutase has been observed, which implies a lower antioxidant defense. This fact, together with the high production of ROS in white adipose tissue, is the cause of the oxidative stress associated with obesity. The increase of oxidative stress, probably due to a deregulated production of adipocytokines, will induce DNA damage that can lead to telomere shortening. In fact, it has been observed that, independently of age, obese women have significantly shorter telomeres than women with a normal BMI (Valdes et al., 2005). This telomere shortening could translate into a significant reduction of years of healthy life and an acceleration of the aging process.

22.2.2.4 Pulmonary diseases

Encompass with the rest of the body, the lungs also age with a progressive reduction of functionality and capacity to respond to environmental stresses and injuries. Traditional age-related pulmonary physiological changes are the dilation of alveoli and the enlargement of the air spaces promoting a decline in lung function and loss of lung elastic recoil (Everaerts et al., 2018; MacNee, 2016). These mechanisms are associated with an accelerated cell senescence process through reduced antiaging biomarkers and shortened telomeres (MacNee, 2016). In this line, a large population study completed in over 45,000 Danish participants identified an association between reduced lung function and shortened telomeres, confirming this theory (Rode et al., 2012). In addition, other factors than age, including disease-specific mechanisms, may influence TL in the lungs. Namely, elevated basal levels of ROS and pro-inflammatory markers also contribute to increasing susceptibility to both acute and chronic pulmonary disease (Janssens et al., 1999).

In patients with respiratory disorders such as COPD, the existence of shorter telomeres in circulating leukocytes has been identified when compared to healthy individuals (Chilosi et al., 2013; Savale et al., 2009). COPD is a good example of an exaggerated inflammatory response frequently described as an accelerated aging process. Patients with this dysfunction exhibit premature cell senescence resulting from both telomere shortening (Rutten et al., 2016; Savale et al., 2009; Tsuji et al., 2004, 2006) and nontelomeric signals such as oxidative stress, inflammation, and sirtuins (Houben et al., 2009; Rutten et al., 2016). Certainly, several mutations in the telomere machinery have been identified in this population with alterations in the telomerase activity present in 1% of patients with COPD (Stanley et al., 2015). Some less conclusive results have been described in airway epithelial cells from patients with COPD, with data favoring shorter (Tsuji et al., 2006),

similar (Birch et al., 2015), or even longer (Everaerts et al., 2018) TL when compared with healthy adults. These discrepancies have been associated with the broad range of comorbidities and disease severity stages that the pathology of COPD encompasses. However, even considering main possible confounders including age, smoking, and lung function, LTL of patients with COPD are significantly associated with increased risk of cancer and total mortality (Lee et al., 2012). Although the exact mechanisms that underlie this relationship are yet unknown, genetic predisposition, oxidative stress, and chronic inflammation have been suggested as potential explanations promoting telomere attrition in COPD (Berndt et al., 2012).

Impaired telomere dynamics have also been identified in circulating cells from patients with other lung disorders including interstitial lung disease (Snetselaar et al., 2015) and idiopathic pulmonary fibrosis (Armanios, 2012). Notably, Ley et al. (2017) observed a relationship between shorter telomeres and increased mortality in patients with chronic hypersensitivity pneumonitis, highlighting the potential role that telomere dysfunction may play in these lung disorders. In addition, mutations in essential telomerase genes including TERT and TERC, have been described in pulmonary fibrosis (Nunes et al., 2014), pulmonary emphysema (Alder et al., 2011), idiopathic pulmonary fibrosis (Armanios, 2012; Snetselaar et al., 2015), and chronic hypersensitivity pneumonitis (Ley et al., 2017).

Despite these compelling early observations of shorter telomeres in both peripheral leukocytes and lung tissue in several pulmonary diseases, future studies need to clarify the potential role that telomeres may play in the pathophysiology of the different lung disorders and the associated mechanisms behind such changes.

22.3 TL and physical exercise

In the last years, many lifestyle interventions have been proposed to modify the speed of telomere shortening, including diet, physical exercise, integrative interventions of lifestyle, improvement of the quality of sleep, etc. Probably, the impact of physical activity has been one of the most studied interventions, and scientific evidence from the last ~50 years has shown that moderate and regular exercise (or physical activity) has many health benefits. These beneficial effects on physiological functions are observed in all age ranges, but its potential role as a healthy-aging intervention is noteworthy.

22.3.1 TL of athletes of different specialties

Physical activity and exercise reduce the risks associated with many age-related diseases. However, when the exercise routines become more demanding in terms of volume (duration of exercise) and/or intensity, the physiological responses may vary. This would be the case for elite athletes. It is well known that acute, high intensity bouts of both aerobic (i.e., running) and resistance exercise can induce different degrees of muscle

damage (Del Coso et al., 2017; Fernandez-Gonzalo et al., 2014), followed by the subsequent inflammatory response (Fernandez-Gonzalo et al., 2012). In addition, production of ROS also occurs during exercise (Thirupathi and Pinho, 2018). These two processes (i.e., muscle damage and regeneration, and the oxidative stress produced by ROS), could have a negative impact on TL. However, as we describe below, this does not necessarily hold true for athletes, due to different beneficial effects of chronic exercise, for example, increased antioxidant capacity. In addition, the exercise discipline (endurance- vs. resistance-type) performed by a particular athlete may influence TL in different organ tissues.

One of the first studies assessing TL in athletes (all of them endurance athletes except one squash player) showed that those participants suffering from exercise-associated fatigue showed shorter muscle TL than their nonfatigue counterparts (Collins et al., 2003). The authors suggested that a greater muscle regeneration process occurred in the fatigue athletes, resulting in more frequent satellite cell proliferation and thus, shorter TL (Collins et al., 2003). When comparing young endurance athletes with subjects exercised at a medium level of activity, Østhus et al. (2012) could not find any difference in muscle TL.

On the other hand, Kadi et al. (2008) studied a population of powerlifter athletes with ~8 years of training experience, reporting no differences in muscle TL between these athletes and a control group. However, they found that heavier loads of training meant a shorter minimum TRF length. This result led the authors to conclude that the maximum tension (e.g., load) sustained by the muscle is an important factor influencing satellite cell recruitment, and thus TL, rather than the total training volume (Kadi et al., 2008). This idea, however, could not be replicated in endurance athletes. Rae and colleagues (Rae et al., 2010) found that the minimum TRF length in skeletal muscle was inversely related to years spent in running and hours of training. Thus, the exercise variables that may affect TL in athletes may differ between resistance- and endurance-type modalities.

Exercise also affects other tissues beyond the skeletal muscle. Therefore, researchers have also investigated the impact of chronic exercise on TL in other cell populations. Using the DNA from saliva samples, Borghini et al. (2015) examined the chronic and acute effects of ultra-distance running in middle-age (~45 years old) athletes. While acute exposure to such an intense bout of running seemed to be detrimental for TL, chronic training had a beneficial effect on TL. Supporting the beneficial role of aerobic exercise, another investigation analyzing LTL found that endurance athletes had longer TL than controls, even after adjusting for age (Denham et al., 2016a,b). These results were similar to the ones found in a population of elite master sprinters (~50 years old), whose training should have been more oriented to power development than to aerobic performance. In any case, LTL was longer in master sprinters when compared with their untrained peers (Simoes et al., 2017). Muniesa et al. (2017) analyzed TL in the most comprehensive population of athletes investigated so far. These authors recruited 61 international-level

athletes from different disciplines, including running events >1500 m, triathlons, weightlifting, judo, gymnastics, canoeing (100–200 m), team sports (basketball and football), and taekwondo, along with 64 healthy nonsmokers, physically inactive controls. They found that athletes had on average higher TL than control subjects, with no effect of sex or age (Muniesa et al., 2017).

In summary, it appears athletes present longer telomeres in circulating cells, but this may not be the case for skeletal muscle. Differences in TL across tissues in this well-trained population may be caused by the divergent effects of exercise in, for example, muscle versus leukocytes. More studies need to address this dichotomy and further investigate whether the exercise discipline (aerobic vs. resistance exercise) influence adaptations in TL, as well as discern the mechanisms behind such changes.

22.3.2 The effect of physical exercise on TL and aging

Within the exercise research area, one of the topics most widely studied is the effects of physical activity on aging, as well as on age-related diseases. Although there is no clear-cut evidence of the capacity of exercise to prolong lifespan in humans, it is now obvious that both resistance and aerobic exercise improve many health-related markers during aging, as well as promote greater quality of life among elderly individuals. Indeed, exercise attenuates most of the major physiological hallmarks of aging (Garatachea et al., 2015). Given the role of telomeres protecting chromosomal integrity, and their alterations with aging (i.e., shortening), it took little time for researchers to investigate whether exercise could modify age-induced telomere shorting and thus, delayed cellular senescence. In this line, observational studies have linked higher levels of physical activity with longer telomeres, especially at older ages (Arsenis et al., 2017).

Although not all studies point in the same direction, it seems the quantity of physical activity may be related to TL in humans. Supporting this idea, an observational study including 69 women and men between 50 and 70 years old, showed that subjects performing moderate levels of physical activity had longer PBMC telomeres, compared with participants with low or very high physical activity level (Ludlow et al., 2008). This inverted "U" relationship between physical activity and TL was later supported in a cohort >200 elderly men (Savela et al., 2013). Thus, it seems that both low and very high levels of physical activity are detrimental to TL during aging. Supporting these findings, Silva et al. (2016) found that TL increased mainly in $TCD8^+$ cells from elderly with intense training lifestyle, whereas TL was increased both $TCD8^+$ and $TCD4^+$ cells in elderly with moderate training lifestyle. Finally, a study assessing >6500 US adults with an age range of 20–80 years old showed that, after adjustments for age, there was a clear dose-response relation between questionnaire-assessed movement-based behaviors and LTL (Loprinzi et al., 2015). However, the real volume and intensity of such movement-based behaviors were not clearly defined.

In 2010, Puterman et al. evaluated TL in postmenopausal women with different stress levels that followed vigorous activity for three successive days. They found an association between higher stress levels and shorter telomeres, and this relationship seemed to be moderated by physical activity (Puterman et al., 2010). In the same year, LaRocca et al. (2010) showed that LTL was greater in endurance-trained elderly compared to sedentary. In this study, authors present two remarkable issues: (1) trained elders did not show differences in LTL compared to trained young people, while telomeres of sedentary elderly were much shorter than those of sedentary youth, and (2) trained youth did not have a higher LTL than sedentary ones (LaRocca et al., 2010).

In all the studies mentioned above, TL was assessed in PBMCs. As previously indicated, the characteristics of skeletal muscle may make the relationship "TL-aging-exercise" unique when compared with other tissues. Skeletal muscle consists of a syncytium of multinucleated muscle fibers that are postmitotic, which would ensure TL remains stable in this nuclei population (with exceptions of rare DNA damage). In contrast, satellite cells undergo divisions after contraction- or injury-induced muscle damage. It is then TL of divided satellite cells that have been incorporated to the muscle fiber, the one that may represent the biological age of muscle, that is, more muscle turnover due to injury or exercise, more satellite cell division, higher a priori reduction in skeletal muscle TL. Yet, moderate and regular exercise may confer positive adaptations not only counteracting potential alterations in TL due to an increased rate of muscle turnover but providing an additional protective environment for telomeres, for example, reducing the concentration of ROS. Indeed, one of the most comprehensive studies investigating TL, aging, and exercise concluded that physical inactivity has a greater effect in muscle senescence than chronological age *per se* (Venturelli et al., 2014). These researchers investigated TL in young, old-mobile and old-immobile subjects in upper- and lower-limb skeletal muscle. They showed that TL was reduced in old subjects, more so in immobile subjects, in skeletal muscle from the lower limbs, but they did not find any difference in TL of skeletal muscle from the upper limbs, despite differences in age (Venturelli et al., 2014). Considering that the lower limbs are in general more prone to suffer from age-induced inactivity, while the arms seem to be used similarly for the activities of daily living across the lifespan (Onder et al., 2005), the results from Venturelli et al. (2014) highlight the important role of physical activity to maintain TL in skeletal muscle with age.

The mechanisms behind any potential beneficial effect of exercise on aging-induced telomere shorting are still far from being completely understood. However, current scientific evidence indicates that there are several factors that may play a role in such adaptations: telomerase activity, oxidative stress, and inflammation. Moreover, it has been suggested that physical activity can preserve skeletal muscle function and mass in older adults through the maintenance of satellite cell telomeres (Kadi and Ponsot, 2010) (Fig. 22.2).

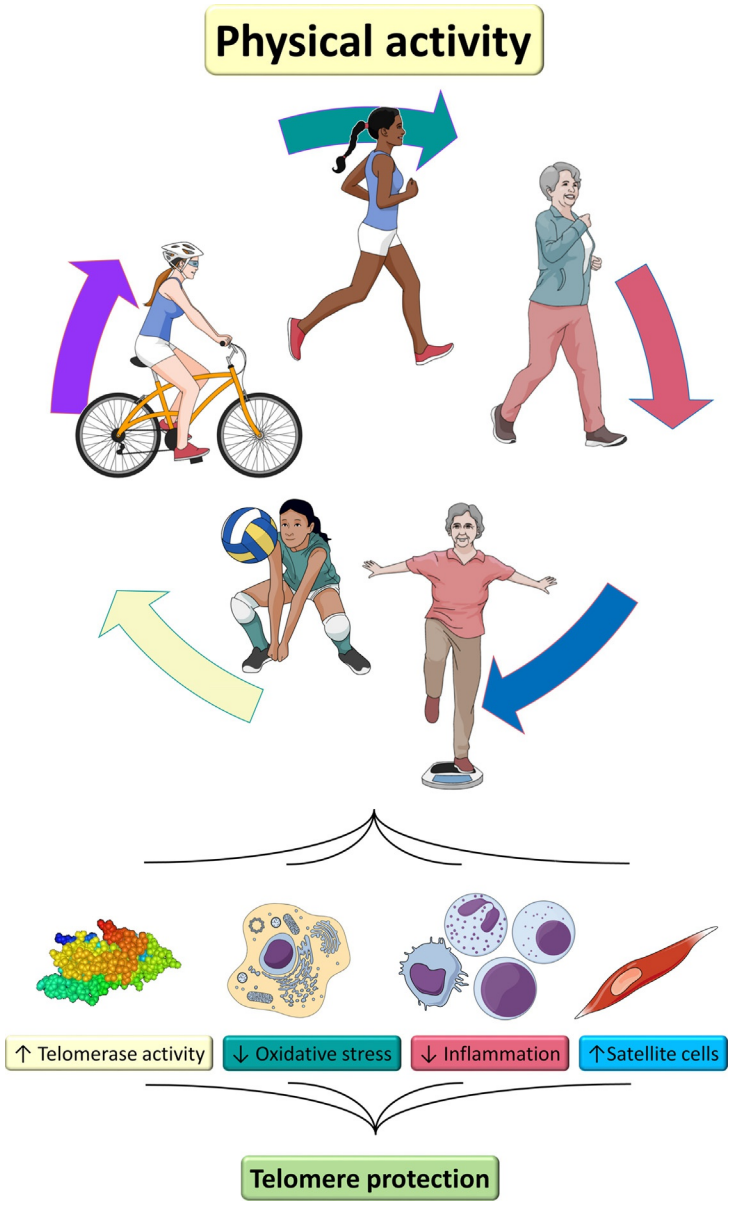

Fig. 22.2 Schematic representation of beneficial effects of physical activity throughout the life of the individual on telomere length and main telomere shortening-associated cellular processes.

22.3.2.1 Telomerase activity

Very few investigations have evaluated the effects of physical activity and telomerase activity in aged individuals. One of the first studies was completed by Werner et al. (2009), showing that both young and aged endurance athletes had increased telomerase activity, as well as telomere-stabilizing proteins levels, compared with untrained controls in PBMCs. Later, Ludlow et al. (2012) described that physical activity restored the significant telomere shortening observed in liver and cardiac tissues from sedentary 1-year-old mice to similar TL than young (8 weeks) mice. However, they found the opposite results in gastrocnemius muscle. Surprisingly, exercise increased telomerase activity in aged gastrocnemius muscle but had a tendency to decrease in aged cardiac and liver tissues.

Telomerase activity can be modified by different mechanisms that would mainly affect the hTERT subunit, including mutations in the promoter, alternative splicing, gene amplification, as well as epigenetic changes. In addition, it has been proposed that hTERT can be autoregulated by telomere looping since it is located very close to the telomere of chromosome 5. It is striking that TERT gene is located near the telomere in long-lived species, but not in short-lived species (Shay, 2016, 2018). In a study that assayed the effects of 30 min of cycling, Cluckey et al. (2017) showed that hTERT expression increased with exercise in both young and elderly subjects, but the magnitude of this increment was greater in the young group, which could mean that hTERT expression is less adaptive to exercise with age.

Finally, in an attempt to relate TL and telomerase activity with exercise training in elderly subjects, Puterman et al. (2018) carried out a study aiming to test the effects of a 24-week aerobic exercise intervention in aged-dwelling dementia caregivers (age range 50–75 years old) who reported high stress and physical inactivity. The results obtained showed that the intervention was able to improve TL, but did not modify telomerase activity.

These findings demonstrate that the mechanisms responsible for the modification of TL exerted by exercise are still elusive and that further research is needed to understand the role of telomerase activity in cells of elderly subjects participating in physical activity programs.

22.3.2.2 Oxidative stress

For many years, the regular practice of physical activity has been considered an effective mechanism to reduce oxidative stress, especially in elderly subjects. This reduction of oxidative damage is associated with lower production of ROS, an improvement in the antioxidant response and less DNA damage, thus offering protection against ROS-induced telomeric shortening (Arsenis et al., 2017; von Känel et al., 2017). Therefore, the relationship between physical exercise and TL seems evident. However, it is important to note that not any intensity of physical exercise is able to prevent or delay

the telomere attrition. In fact, the effect of exercise on antioxidant responses seems to follow the phenomenon of "hormesis" (Radak et al., 2008) and adapt to a curve with a form of "U" (Ludlow et al., 2008; Savela et al., 2013). So, exercise protocols of moderate intensity are the most suitable to induce beneficial adaptations for the organism (Radak et al., 2008).

In this context, although there are studies that do not find an association between habitual physical activity and LTL (Shin et al., 2008), most of the scientific evidence point to a direct relationship between the regular exercise of moderate intensity and longer telomeres. As previously mentioned, in general, it is accepted that the improvement of the oxidative status, together with a decrease in the inflammatory response, could be the cause of the positive association between exercise and the size of telomeres (Mundstock et al., 2015; Arsenis et al., 2017). In addition, in middle-aged athletes exercise has also been associated with an increase in the expression of DNA repair enzymes (e.g., Ku proteins), an overexpression of telomere protective proteins (e.g., telomeric repeat-binding factor 2) and a decrease in the regulation of cell cycle proteins, such as p16, a protein that prevents the progression from G1 to S phase of cell cycle (Werner et al., 2009).

22.3.2.3 Inflammation

Over the past decades, there have been many studies aimed at establishing the relationships between physical activity and inflammation in aging (Rodriguez-Miguelez et al., 2014, 2015; Mejías-Peña et al., 2016, 2017). However, very few have tried to analyze its relationship with telomere shortening.

In the study of LaRocca et al. (2010), which showed a higher LTL in endurance-trained elderly compared to sedentary individuals, no variations in CRP concentrations were found. Similarly, Silva et al. (2016) reported that TL increased in $TCD8^+$ cells from elderly with intense training lifestyle and in both $TCD8^+$ and $TCD4^+$ cells in elderly with moderate training lifestyle. However, no changes were observed in circulatory inflammatory markers of IL-8, IL-6, and IL-10 or in cytokine levels (IL-2, IL-6, TNF-α, IFN-γ, and IL-10) from stimulated-PBMCs culture supernatants when comparing both intense and moderate training lifestyles with elderly who never trained. Conversely, Cluckey et al. (2017) described an increase in IL-6 gene expression in both young and old subjects after 60 min of cycling similar to the hTERT response. Finally, Puterman et al. (2018), even found an improvement in TL from elderly subjects with 24-week aerobic exercise.

Attempts have been made to explain the interaction between inflammation and telomerase activity in different ways. Particularly, all theories converge on IL-6, a myokine that could also explain how physical activity modifies telomerase activity. It is known that TNF-α and IL-6 can activate NF-κB, so that p65, by direct binding with hTERT, promotes its translocation to the nucleus inducing telomerase activation. Moreover, it has

been described that IL-6 has three downstream targets that would interact with hTERT subunit by modifying telomerase activity, namely signal transducer and activator of transcription 3 (STAT3), extracellular signal-regulated kinase (ERK)/mitogen-activated protein kinase (MAPK), and serine/threonine kinase (Akt)/NF-κB. Therefore, activated STAT3 binds hTERT promoter stimulating hTERT upregulation and ERK/MAPK cascade-mediated histone H3 phosphorylation and acetylation can enhance transcriptional activation of hTERT. Akt induces hTERT phosphorylation, which increases telomerase activity (Deng et al., 2016).

Even considering the exercise-associated changes on inflammatory markers reported in elderly subjects, further studies are required to determine the exact crosstalk between inflammation and telomere shortening in this population.

22.3.2.4 Satellite cells

Satellite cells are a specific type of muscle cells located between the basal lamina and the plasma membrane of the muscle fiber. These precursor cells of skeletal muscle fibers are activated as a consequence of the natural process of muscle regeneration, or when muscle injury occurs. Notably, there are a considerably lower number of satellite cells in the elderly. In fact, human studies show that, after 70 years, there is a significant decrease in the amount of this cell type (Kadi and Ponsot, 2010). Some studies have associated this reduction with the loss of muscle mass observed in sedentary individuals. Specifically, it has been estimated that in sedentary elderly the loss of muscle tissue is approximately 40% compared with younger counterparts (Sharples et al., 2015).

Although there are many factors affecting the muscle deconditioning seen in the elderly, TL seems to have an important role in the maintenance of satellite cells, and thus in skeletal muscle integrity. In fact, the study carried out by Werner et al. (2009) revealed a positive correlation between TL and the number of satellite cells of skeletal muscle in elderly women. However, other study reported that biological aging of skeletal muscle is not associated with TL but, possibly, with other factors that could also modify the amount of satellite cells available during muscle regeneration processes (Renault et al., 2002). Like other modifiable factors related to lifestyle, regular physical activity, in the form of both aerobic and resistance exercise, has been proposed as an effective mechanism to counteract the decline of skeletal muscle in the elderly, probably by stimulating the accumulation of satellite cells (Kadi and Ponsot, 2010; Rebelo-Marques et al., 2018). In this sense, physical exercise may be related to TL preservation (see "The effect of physical exercise on TL and aging" section). Indeed, greater telomer shorting may be behind the decreased replication capacity of satellite cells seen in subjects with low levels of physical fitness (Meyer et al., 2016).

22.3.3 The effect of physical exercise on TL and aging-associated diseases

Throughout the present chapter, evidence has been presented on the positive correlation between regular physical activity and TL. Furthermore, it is also known that the practice

of regular exercise not only decreases the risk of developing age-related diseases, but it is able to attenuate and manage the specific disease symptoms. The following section aims to analyze the interconnection between the three factors: physical exercise, TL, and age-associated diseases.

22.3.3.1 Cancer

For years, experts have shown that regular physical activity can reduce the risk of developing certain types of cancer. The strongest association is established between physical inactivity and the risk of breast, colon, prostate, endometrial, and lung cancer. Along with smoking, lack of physical activity is one of the risk factors for cancer that can be modified by changes in lifestyle (World Cancer Research Fund and American Institute for Cancer Research, 2007). The American College of Sports Medicine recommends regular exercise, between 30 and 60 min per day, as it not only reduces the risk of developing cancer but may delay aging. Specifically, women who walk quickly between one and two half hours per week have a 10%–25% reduced breast cancer risk (Wu et al., 2013). In fact, although exercise recommendations to reduce breast cancer are not fully established, observational studies suggest that it may be dose-dependent, that is, a longer duration or higher intensity of physical activity could be associated with higher protection (Lynch et al., 2011; Mijwel et al., 2018).

Various biological mechanisms have been postulated to explain the association between physical exercise and cancer. Thus, some potential factors could be changes in the concentration of sexual and metabolic hormones and growth factors, decrements in central adiposity and obesity, and possible changes in immune function and inflammatory response. In addition, in the last years, other factors such as oxidative stress, global DNA hypomethylation, vitamin D exposure or TL also seem to be involved (Neilson et al., 2013). Regarding studies on TL in cancer patients who practice exercise, it has been shown that exercise intensity can be an important determinant of TL. Indeed, postmenopausal women with stages I–III breast cancer who participated in a moderate or vigorous exercise program had significantly longer LTL than sedentary patients (Garland et al., 2014). Similarly, Ornish et al. (2013) reported that, in patients with low-risk prostate cancer, changes toward a healthier lifestyle, including a higher level of physical activity, also correlated positively with increased telomerase activity and an increase in TL.

Importantly, there are exercise programs specifically designed for cancer patients or survivors. However, experts highlight that the key to success lies in the individualization of the exercise, gradually accommodating the exercise program to the special needs of each patient. In any case, physical activity improves functional capacity and body composition, reduces side effects of treatment, ameliorates the mood and personal image of patients with cancer, and seems to counteract the changes in biomarkers that induce carcinogenesis (Stefani et al., 2018).

22.3.3.2 Cardiovascular diseases

If to date there are few studies that have analyzed the association between physical exercise and TL in the elderly, the number of investigations is considerably reduced when it comes to establishing the association between both parameters in elderly with chronic cardiovascular diseases. In general, it is accepted that the practice of regular physical activity can be an effective therapeutic tool to prevent or delay the onset of age-associated cardiovascular diseases. In fact, there are multiple pieces of evidence that indicate that patients with hypertension, coronary disease, peripheral arterial disease, or arteriosclerosis can obtain important benefits for their health if they undergo physical training programs (Sallam and Laher, 2016). This protective effect of physical activity could be due to a decrease in risk factors (blood pressure, obesity, and lipid profile). At the molecular level, although the mechanisms have not been fully established, factors such as a decrease in oxidative stress and inflammation could be involved in the beneficial effect of exercise, which could possibly also result in the maintenance of telomere integrity (Denham et al., 2016a,b). Thus, studies in mice showed a higher expression of TRF1 and TRF2, telomere protective genes (POT), and proteins that respond to DNA damage that are transiently associated with telomeres, such as Ku70/80, CHK2, and p53. These results were observed after 1 year of wheel-running exercise. These adaptations could contribute to the maintenance of telomeres, attenuating the age-associated loss of TL in the cardiac muscle of animals (Ludlow et al., 2017). These data support findings from Werner et al. (2008) in cardiomyocytes of mice; the authors demonstrated that long- and short-term voluntary physical exercise upregulated cardiac telomerase activity, increased TERT and TRF2 expression, and downregulated the expression of the aging marker protein p16.

The research carried out by Denham et al. (2013) comparing ultramarathon runners and control subjects demonstrated that ultra-resistance aerobic exercise was associated with less telomere attrition, independently of age or cardiovascular risk factors present in the participants. Regarding the elderly population, there are no studies where an intervention based on physical exercise has been conducted to determine its effect on TL in subjects with cardiovascular diseases, limiting the works found to cardiovascular risk factors. Thus, an observational study by Shadyab et al. (2017) with 1476 older women, showed that those who performed moderate to vigorous physical activity and who walked more quickly had a longer LTL. These results were maintained even when the models were adjusted for the different diseases that each participant presented (including coronary heart disease and stroke). It is important to note that, in some of these studies, physical activity was measured either in a self-declared manner or using pedometers, which may potentially represent a limitation to consider (Peng et al., 2017; Shadyab et al., 2017). Therefore, it is necessary to conduct further studies addressing the effects of physical exercise on TL and aging-associated cardiovascular diseases.

22.3.3.3 Metabolic disorders

It is known that both caloric restriction and exercise can have beneficial effects on oxidative stress and inflammation and, consequently, on the maintenance of telomere integrity. However, there are few scientific studies that specifically assess the effect of physical exercise on TL in elderly people with obesity. In this regard, Mason et al. (2013) demonstrated that 12 months of combined treatment of diet and aerobic exercise was not enough to significantly modify TL in postmenopausal women. The same result was obtained in a 4.5-year follow-up study based on a healthy eating program and an increase in physical activity in 522 subjects with impaired glucose tolerance; after the intervention, there were no differences in TL between experimental and control groups (Hovatta et al., 2012).

The prevalence of T2D among the elderly population is high and there are numerous studies that have correlated higher insulin sensitivity with the practice of aerobic exercise in all age groups, including the elderly (Honka et al., 2016). In contrast, shorter telomeres have been associated with an increased risk of cardiovascular and metabolic diseases, such as T2D. In this line, a study including 35 elderly women between 69 and 74 years showed that endogenous glucose production was reduced after 4 months of resistance training. In parallel, an improvement in glycemic control was observed. In turn, these metabolic changes were associated with an elongation of telomeres, which could indicate a delay in the cellular aging process; the authors suggest that, if physical training is able to induce a significant improvement in glucose homeostasis, this could result in a preservation of TL, or even an elongation of the telomeres and, ultimately, a lower risk of suffering from T2D or cardiovascular diseases (Honka et al., 2016).

22.3.3.4 Pulmonary diseases

Considering the previously described associations between pulmonary dysfunctions and reduced TL and the positive effects that physical activity exerts in counteracting cellular senescence, it would not be surprising that patients with respiratory disorders will get cellular benefits from an active lifestyle. In this regard, Østhus et al. (2012) described the upregulation of different proteins involved in telomere protection, including TRF2 and Ku proteins, in physically active middle-aged adults when compared with age-matched sedentary participants. Emerging evidence also suggests that other cellular aging mechanisms involved in the pathogenesis of COPD such as reduced sirtuin 1 (Paschalaki et al., 2013; Rutten et al., 2016; Yanagisawa et al., 2017) are able to significantly increase after exercise training (Ferrara et al., 2008; Huang et al., 2016). However, the existing literature provides very limited experimental data on the effects of physical activity and telomere attrition specifically in pulmonary disorders.

Several potential mechanisms associated with cellular senescence and shorter TL (e.g., oxidative stress, inflammation) commonly observed in different lung disorders (Cantin et al., 2015; Murdoch and Lloyd, 2010; Oudijk et al., 2003) may benefit from frequent

physical activity. Especially, telomerase seems to be involved in modulating the activity of the NF-κB and thus, systemic inflammation (Zhang et al., 2016). Indeed, circulating concentrations of IL-6 have been inversely associated with TL in patients with COPD (Savale et al., 2009). Similarly, shortened telomeres in COPD seem to be related to reduced activity of the antioxidant enzyme superoxide dismutase (Houben et al., 2009). Given the reliance between physical activity and associated reductions in both oxidative stress and inflammatory markers (Rodriguez-Miguelez et al., 2014, 2015; Wang et al., 2014), one would expect that the regular engagement in different physical activities of patients with pulmonary disorders would attenuate cellular senescence and telomere attrition. Unfortunately, no studies have yet evaluated these potential relationships, and all the hypothesis are based on mere speculations. Therefore, further research is needed to clarify this potential relationship and evaluate if patients with lung diseases would benefit at both physiological and cellular level from an active lifestyle.

22.4 Conclusions

Telomere shortening is one of the more widely accepted theories of aging within the scientific community. However, age-induced telomere attrition seems to correlate with other cellular processes such as telomerase activity, oxidative stress, or sustained low-grade inflammation. Although there are discrepancies, it seems that all these mechanisms are also altered in the aging-associated pathological processes.

Lifestyle changes, including an increase in physical activity, can delay the appearance of the aging phenotype and associated comorbidities, preventing or delaying the onset of diseases such as cancer and certain cardiovascular, pulmonary, and metabolic disorders. In fact, most researchers suggest that regular exercise, performed with adequate duration and intensity, could induce beneficial adaptations for the organism and counteract telomere shortening through its proven protective and restorative effects as an antioxidant and antiinflammatory tool. However, further intervention studies are still needed in order to understand the impact of physical activity on TL and the relationship with oxidative stress and inflammation. For this purpose, it would be advisable to use different protocols and exercise intensities, with special emphasis on those subjects who have age-associated chronic diseases. The final outcome of such studies should be to prescribe exercise protocols specially designed for the characteristics and needs of the elderly population, increasing the beneficial adaptations and, at the same time, reducing any potential undesirable side effects.

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CHAPTER TWENTY-THREE

Proteomics and metabolomics research in exercise and sport

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23.1 OMICS in sports

The advancement in proteomics and metabolomics tools including mass spectrometry (MS) technologies and bioinformatics resources has offered a unique opportunity to complement genomics and transcriptomics data generated in the recent years (Tanaka et al., 2016). The integration of OMICS technologies has provided a comprehensive coverage of the molecular pathways involved in complex physiological and pathological functions (Tyers and Mann, 2003; Misra et al., 2018), paving the way for a more precise monitoring of the molecular changes associated with athletes' health, training and recovery from injury. These changes reflect complex interactions between genetic predisposition and environmental factors such as exercise and diet that together determine athletic performance. Monitoring these molecular changes associated with athletes from different sports has provided invaluable tools for biomarker discovery and validation, aiming for optimal balance between training and recovery for every athlete (Al-Khelaifi et al., 2018a).

Proteomics and metabolomics have provided deep phenotypes for complex traits including changes in peptides, proteins, and various end products (metabolites) underlying the molecular pathways of their cellular processes (Kelly et al., 2015). In sports science, proteomics and metabolomics offer a quantitative measurement of the metabolic profiles of professional athletes for the identification of selective biomarkers (Wang et al., 2016). These molecular biomarkers can include small molecules, proteins, and metabolites that reflect the specific diet (nutrition, supplements, and drugs) and exercise (energy, fatigue, and injury) of an athlete. Monitoring selected biomarkers could reveal potential health issues, therefore helping athletes to decide the optimal time and duration of their training and participation in sports events (Lee et al., 2017). Biomarker monitoring during recovery from injury or competitive events could also identify hidden metabolic issues that require a special attention and treatment (Lee et al., 2017). Identification of novel and consistent molecular signatures of blood manipulation is a subject of interest

Sports, Exercise, and Nutritional Genomics
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in antidoping research seeking a complementary test to be used in combination with athletes blood passport or even introduced as a stand-alone test (Wang et al., 2017).

This chapter aims to cover known and potential biomarkers that reflect the response of skeletal and cardiovascular muscles to exercise as well as metabolic signatures of different sports grouped as power or endurance sports. These protein and metabolic biomarkers represent the response to athletes' nutrition, exercise, metabolic health, energy demands, and recovery from injury. Evidence-based examples of biomarkers for different features of health and performance are reviewed, aiming at defining an improved approach for sport assessment and coaching to improve health, performance, and recovery of athletes.

23.2 Exercise and sports-related proteomics research

Investigation of the metabolic pathways associated with exercise-driven muscular and cardiovascular adaptation has gained increased interest due to its importance to training, health improvement, and disease prevention (Fiuza-Luces et al., 2018). Changes in human proteome in response to exercise are essential part of these adaptations as structural proteins and metabolic enzymes are instrumental players in this process (Burniston and Hoffman, 2011). The recent advancement in proteomic techniques has made protein analysis possible in complex biological matrixes such as plasma and tissue that are often utilized in exercise research. Since human muscle biopsies are difficult to obtain, most research was conducted in animals including rodents, equines, swine, and fish among others (Richardson et al., 2009; Martin-Perez et al., 2012; Scoppetta et al., 2012). MS can identify proteins based on their molecular ionization (Aebersold and Mann, 2003), therefore providing quantitative and qualitative proteomic analysis. Conventional proteomics techniques such as one- and two-dimensional polyacrylamide gel electrophoresis were initially utilized in exercise research. More recently, advanced techniques such as differential gel electrophoresis (DIGE) and gel-free techniques (ICAT, SILAC, and iTRAQ) have improved research outcome and allowed more comprehensive coverage of human proteome and strengthened its quantitative analysis (Petriz et al., 2012). A more novel proteomic approach that combined SILAC with iTRAQ reagents was utilized, thus improving assessment of protein turnover (Jayapal et al., 2010). Despite emergence of conventional and advanced proteomics techniques, their implementation in exercise research is still at an early stage. This section provides a summary of the current proteomic exercise research and utilized models in the study and investigation of exercise physiology.

23.2.1 Proteomic responses of skeletal and cardiac muscle to exercise

Biological systems are characterized by dynamic interactions among proteins and polypeptides (Graves and Haystead, 2002; Hirsch et al., 2004). Proteomics offers

quantification of proteins involved in these pathways at baseline and in response to various biological perturbations (Anderson and Anderson, 1998). Proteomics also addresses the posttranslational modifications that can define and shape the function of these proteins (Vercauteren et al., 2007). Exercise is an example of such perturbation that can alter protein expression and posttranslational modification by inducing various signaling pathways with impact on protein regulation and enzyme activity. The plasticity and adaptation of skeletal and cardiac muscles to exercise is a topic that is largely studied because of its importance in exercise physiology and health protection (Egan and Zierath, 2013; Ventura-Clapier et al., 2007). Previous studies have shown a direct relationship between intensity of exercise and adaptation of the skeletal muscles (Holloszy, 1975; Booth and Thomason, 1991; Safdar et al., 2009), modulation of the cardiovascular system (Longhurst et al., 1981; Fenning et al., 2003; Diffee, 2004), and reduction of pathology (Lima et al., 2008; Powers et al., 2008). Novel pathways underlying the benefits of training were uncovered through studying the impact of exercise in skeletal and cardiac muscles (Burniston and Hoffman, 2011). Contractile proteins are responsible for muscle hypertrophy and atrophy in response to exercise and weight loss, respectively (Isfort et al., 2002a, b; O'Connell et al., 2007). Exercise modulates various molecular signaling pathways that include different kinases and phosphatases causing modulation of protein expression (Nader and Esser, 2001; Coffey et al., 2007). The positive impact of different forms of exercise (interval, acute, and chronic) with variable intensities (low, moderate, and severe) on muscle growth has been verified by many groups (Booth and Thomason, 1991; Holloszy, 2005).

23.2.1.1 Proteomics of exercising skeletal muscle

Skeletal muscle plasticity in response to exercise has gained a wide interest due to the functional role of skeletal muscles in athletic performance (Petriz et al., 2017). Exercise-triggered adaptations in skeletal muscle include changes in contractile proteins (Adams et al., 1993), mitochondria functions (Spina et al., 1996), metabolic pathways (Green et al., 1992), and gene expression (Pilegaard et al., 2003). Skeletal muscle works as an endocrine organ that controls the secretion of various growth factors and myokines in response to exercise to regulate inflammation, muscle hypertrophy, and cell metabolism (Raschke et al., 2013). Proteomic analysis of skeletal muscle secretome is essential to identify biomarkers of exercise-associated tissue injury and muscle remodeling (Scheler et al., 2015). Moreover, the proteomics analysis can be performed using in vitro exercise models to investigate the molecular mechanisms underlying muscle contraction (Henningsen et al., 2010).

The adaptations of skeletal muscle to training is influenced by its duration, intensity, and frequency (Egan and Zierath, 2013). However, studies of skeletal muscle proteomics in response to exercise have been somewhat limited. Table 23.1 summarizes some of these studies, highlighting the type of exercise and list of altered proteins in response

Table 23.1 A list of proteomics studies of exercising skeletal muscle in animal models and humans

Model	Exercise	Finding	Reference
Rat gastrocnemius muscle	High-intensity exercise for 3 min with/out 30 min of recovery	Changes in heat shock protein 20 kDa, creatine kinase, troponin T, and adenylate kinase	Guelfi et al. (2006)
Rat plantaris muscle	Chronic endurance exercise (70%–75% VO_2 peak) for 30 min, 4 days per week for 5 weeks	80 proteins corresponding to 40 gene products suggesting a shift from glycolysis toward greater fatty-acid oxidation	Burniston and Hoffman (2011)
Rat epitrochlearis muscle	High intensity exercise (14×20-s swimming exercise bouts carrying a weight with 10-s pause between bouts)	Five mitochondrial enzyme proteins were altered including the ATP synthase β-subunit, NADH dehydrogenase Fe-S protein 1, 75 kDa, NADH dehydrogenase Fe-S protein 2, oxoglutarate dehydrogenase and mitochondrial malate dehydrogenase	Yamaguchi et al. (2010)
Rat tibialis anterior and soleus muscles	One hour exercise for 3 times a week using rodent treadmill at 10% slope	A downregulation of several glycolitic enzymes and a high basal level of carbonylation in proteins involved in energy metabolism, muscle contraction, and stress response	Magherini et al. (2012)
McArdle mouse (glycogen deficiency) quadriceps muscle	Endurance exercise	Induction of expression of LIM and calponin homology domains-containing protein 1 (LIMCH1), poly (ADP-ribose) polymerase 1 (PARP1), tigger transposable element derived 4 (TIGD4) and mitogen-activated protein kinase 12 (MAPK12)	Fiuza-Luces et al. (2018)
Mouse gastrocnemius muscle	Regular exercise in mice fed with diet high in dietary fats (HFD)	(MUP1) decreased after HFD but increased with exercise in an AMPK-dependent manner	Kleinert et al. (2018)

Table 23.1 A list of proteomics studies of exercising skeletal muscle in animal models and humans—cont'd

Model	Exercise	Finding	Reference
Transgenic mice expressing ALDH2	Intensive exercise (a motor-driven rodent treadmill for 7 days at gradually increasing speeds from 20 to 40 m/min at a 5% grade)	Changes in manganese superoxide dismutase and NAD(P)H:quinone oxidoreductase 1	Zhang et al. (2017)
77 healthy and sedentary men and women (vastus lateralis biopsies)	Moderate endurance training (16 weeks with stationary bicycle, lasting 20 min at an intensity eliciting 70% of the peak heart rate)	Myosin heavy chain (MHC) isoforms type II was reduced and MHC I was increased	Short et al. (2005)
Ten recreationally active subjects	Acute and chronic exercise that included 20-km time trial on a cycle ergometer	Increasing protein stability	Hinkley et al. (2017)
Vastus lateralis muscle biopsies from five human subjects	Interval training (motorized treadmill, with a 6-week interval-training program, 30 min duration, three times a week with a day's rest in between)	Increased succinate dehydrogenase, trifunctional protein-alpha and ATP synthase alpha- and beta-chains	Holloway et al. (2009)
Vastus lateralis muscle from human subjects	Exercise at high altitude (4000 m)	Enhanced mitochondrial and angiogenic protein expression associated with reduction in intramuscular oxygen content below 1% (8 torr), while HIF-1alpha is stabilized	Flueck (2009)
Vastus lateralis muscle samples from healthy men	Two bed rest campaigns, one lasting 35 days and one 24 days	Down regulation of myofibrillar proteins, metabolic enzymes and antioxidant defense systems both post-8 days and post-35 days of bed rest	Brocca et al. (2012)
Meta-analysis (four studies) of response of skeletal muscle to exercise vs obese/T2DM	Endurance training	Upregulated proteins in exercised muscle include NADH ubiquinone oxidoreductase subunits A8 (NDUA8), B8 (NDUB8) and S2 (NDUS2); glutamic-oxaloacetic transaminase 2 (AATM) and ATP synthase beta (ATPB)	Srisawat et al. (2017)

to exercise. A proteomics study comparing rat gastrocnemius muscle at resting state against high intensity exercise for 3 min followed by 30 min recovery revealed exercise-driven over expression of heat shock protein 20 kDa, creatine kinase, troponin T, and adenylate kinase out of 61 identified proteins (Guelfi et al., 2006). Another study focused on proteomics analysis of rat plantaris muscle adaptation in response to chronic endurance exercise (70%–75% VO_2 peak) of 30 min/day for 5 weeks. Compared to resting controls, the study revealed that endurance exercise increased the cardiovascular capacity by 14% of VO_2 peak, together with changes in the expression of 15 proteins related to metabolic adaptations such as glycolysis and the fatty acid (FA) oxidation (Burniston, 2008).

A study investigating the response of rat epitrochlearis muscle following high-intensity intermittent exercise (4 bouts of 20 s swimming for 5 days with 15% overweight) revealed changes in 13 proteins including two novel ones (NDUFS1 and parvalbumin) (Yamaguchi et al., 2010). Furthermore, the proteomics and protein carbonylation in trained and untrained rat tibialis anterior (fast-twitch fibers) and soleus (slow-twitch fibers) muscles were investigated to understand the relationship between physical exercise, reactive oxygen species, and skeletal muscle modification in response to appropriate or inappropriate exercise. The study identified a down-regulation of several glycolytic enzymes and a high basal level of carbonylation in several proteins involved in energy metabolism, muscle contraction, and stress response in all animals and in both types of skeletal muscle (Magherini et al., 2012). McArdle mouse model that suffers from deficiency in glycogen was used to study proteomic changes in muscle tissue induced by endurance exercise training with and without glycogen availability. Proteomics of the quadriceps muscle in response to endurance exercise (8 weeks' moderate-intensity treadmill training) identified changes in three proteins common between wild type and McArdle mouse including LIM and calponin homology domains-containing protein 1 (LIMCH1), poly (ADP-ribose) polymerase 1 (PARP1), and tigger transposable element derived 4 (TIGD4). McArdle mice showed also a high expression of mitogen-activated protein kinase 12 (MAPK12), suggesting that glycogen availability affects muscle protein signaling adaptations to endurance exercise training (Fiuza-Luces et al., 2018). A proteomics characterization of skeletal muscle following exercise in mice fed with diet high in dietary fats (HDFs) has revealed that major urinary protein 1 (MUP1) decreased after HFD but increased with exercise in an AMPK-dependent manner. MUP1 also triggered GLUT4 translocation in cultured muscle cells. The study concluded that MUP1 was a diet and exercise-regulated skeletal muscle protein that enhances insulin sensitivity in muscle cells (Kleinert et al., 2018). A more recent study of transgenic mice expressing aldehyde dehydrogenase (ALDH2) suggested that ALDH2 can repair exhaustive exercise-associated mitochondrial dysfunction in skeletal muscle by mitochondria dynamic remodeling and improving mitochondrial quality (Zhang et al., 2017).

Fewer studies examined the proteomics of exercising human skeletal muscle because of the invasive nature of sample collection. In a study that included 77 healthy and sedentary men and women, muscle biopsies were obtained before and after 16 weeks of bicycle training (45 min at 80% peak heart rate, 3–4 days/week). Moderate endurance training induced variation of skeletal muscle's myosin heavy chain (MHC) isoforms (Short et al., 2005). Another study investigating the effects of acute and chronic exercise on skeletal muscle proteome in 10 recreationally active subjects following 12-day cycling protocol revealed that short-term intense exercise created a cellular environment resistant to exercise-induced stress by increasing protein stability (Hinkley et al., 2017). A study of vastus lateralis muscle biopsies from five human subjects who underwent 6 weeks of interval training has revealed changes in 20 proteins with increased cardiovascular capacity (7%) including mitochondrial components succinate dehydrogenase, tri-functional protein-alpha, and adenosine triphosphate (ATP) synthase alpha- and beta-chains (Holloway et al., 2009). Extended resting periods are associated with lower limb atrophy (Leblanc et al., 1992). Proteomics of bed-rested muscle fibers has revealed that muscular atrophy is associated with lower MHC distribution and reduced thin filament proteins, as well as with elevated type I and reduced type IIA fibers in vastus lateralis and soleus muscles. The proteomic alterations were also associated with reduced levels of mediators of aerobic metabolism and antioxidant proteins but increased levels of mediators of anaerobic glycolysis such as glycogen phosphorylase and α-enolase (Leblanc et al., 1992; Brocca et al., 2012). In response to exercise, myofibrillar protein isoforms exhibit changes in MHC and thin filament isoforms as well as in various plasma calcium and mitochondrial proteins. Changes include structural proteins, enzymatic proteins (such as pyruvate dehydrogenase complex, fumarate hydratase, malate dehydrogenase, and aspartate aminotransferase), and components of electron transport chain (such as complex I, IV, and ATP synthase subunits) (Holloway et al., 2009; Gondin et al., 2011; Hody et al., 2011). Investigation of changes in skeletal muscles proteomics in response to exercise at high-altitudes identified mitochondrial autophagy and protein degradation as the prime adaptive mechanisms to altitude exposure (Flueck, 2009). A meta-analysis comparing alterations in skeletal muscle proteins of obese/T2DM individuals and endurance-trained individuals showed differences in proteins related to complex I of the electron transport chain as a major mechanism of exercise-induced protection against obesity/Type 2 diabetes deleterious effects (Srisawat et al., 2017).

23.2.1.2 Proteomics of exercising heart muscle

Heart disease is a leading killer worldwide, therefore the heart muscle has been the focus of biomedical research for decades (Gaziano et al., 2010). Exercise plays an important role in modulating the cardiovascular system and lowering the risk of heart disease (Powers et al., 2008; Xu et al., 2008). Endurance training triggers cardiac

hypertrophy (Frey and Olson, 2003; Diffee, 2004), but research into molecular changes of heart tissue after intense exercise is still limited. A study of rat heart proteome has shown that 6 weeks of exercise caused ventricular hypertrophy associated with upregulation in six protein spots (Boluyt et al., 2006). Intensive swimming training of rats for 8 weeks caused 20% increase in their heart muscle, associated with differences in 11 protein spots related to metabolic pathways of oxidative and contractile functions (Sun et al., 2008). The upregulated contractile proteins increase cardiac output and muscle hypertrophy. Chronic endurance exercise of rats showed modulation of left ventricle proteome and improvement of rat cardiovascular capacity associated with changes in 23 proteins mostly related to myofibrillar and miscellaneous metabolic processes (Burniston, 2008).

Investigation of the subsarcolemmal and intermyofibrillar mitochondrial subpopulation proteins isolated from the myocardium of sedentary and trained rat (70% VO_2 peak) identified changes in 13 proteins that are proposed to offer cardioprotective role (Kavazis et al., 2009; Bansal et al., 2010). Further investigation looked at proteome modulation of infarcted rat left ventricle in response to 8 weeks of exercise and identified 20 protein alterations including glutathione peroxidase, manganese super peroxidase, and voltage-dependent anion-selective channel-2, suggesting modulation of apoptotic and antioxidant stress within exercising myocardium (Kavazis et al., 2009; Bansal et al., 2010). Another study has identified specific differences in cardiac left ventricle tissue proteome between nonselectively bred rats with unique inherent exercise abilities. Protein profiling revealed differences between rats with high- and low-running performance in 15 proteins involved in metabolism, oxidative-stress scavenging mechanisms, microfibrillar, and cytoskeletal structure. The enhanced contractile protein concentrations in the high-running performance rats contributed to their greater exercise capacity, whereas levels of proteins that could mark higher cardiovascular risk were higher in low performance rats (Ribeiro et al., 2018). Investigation of changes in the cardiac proteome of rainbow trout during development of a typical exercise regimen revealed that early training-associated structural remodeling was accompanied by an elevation in levels of specific proteins involved in muscle contraction and integrity. The study also revealed reduced levels of energy proteins with exercise, suggesting that regulation of energy pathways is independent of protein abundance (Dindia et al., 2017). Changes in cardiac phosphoproteome to a standard exercise in male rats revealed novel phosphorylation sites including four cardiac proteins and seven kinases such as myofibrillar protein kinases (Guo et al., 2017). A moderate treadmill exercise of rats for over 1 year was shown to alter the levels of proteins involved in carbohydrate oxidation-based metabolism including 17 heart mitochondrial phosphorylation sites that act on kinases such as RAF and p38 (Ferreira et al., 2014). Table 23.2 summarizes some proteomics studies of cardiac muscle, highlighting the type of exercise and list of altered proteins in response to exercise.

23.2.1.3 Exercise and proteomic research in blood

Investigations of serum proteome changes in response to exercise have been somewhat limited because of its complex composition and difficulty in interpreting data. Previous work has reported 18 serum proteins modulated by 12 weeks exercise including an increase in complement factor H involved in protection from microangiopathy and macular degeneration (Yang et al., 2010). Profiling of circulating peptides/proteins induced by 1-year moderate-intensity aerobic exercise training (70% of maximal heart rate,

Table 23.2 A list of proteomics studies of exercising cardiac muscle in animal models

Model	Exercise	Finding	Reference
Rat	Exercise training on treadmill (6 weeks of progressive treadmill training 5 days/week)	Increase in 20 kDa heat shock protein (hsp20) that persisted for at least 72 h of detraining	Boluyt et al. (2006)
Rat	Chronic exercise training (8 weeks of swimming training 7 days/week)	Alterations in proteins associated with the mitochondria oxidative metabolism, such as prohibitin, malate dehydrogenase, short-chain acyl-CoA dehydrogenase, triosephosphate isomerase, electron transfer flavoprotein subunit beta, ndufa10 protein, ATP synthase subunit alpha and isocitrate dehydrogenase [NAD] subunit	Sun et al. (2008)
Rat	Endurance exercise training (gradual increase in running time, beginning with 10 min/day and ending with 50 min/day)	Alterations in glutathione peroxidase, and manganese superoxide dismutase	Kavazis et al. (2009)
Rat	Post-myocardial infarction endurance exercise on a motorized rodent treadmill for 8 weeks	Decreased levels of voltage-dependent anion-selective channel 2 and increased levels of glutathione perioxidase and manganese superoxide	Bansal et al. (2010)
Rat	Endurance exercise on treadmill (running once a day for 5 consecutive days)	Alterations in aldehyde dehydrogenase 2 (ALDH2), α-crystallin B chain and heat shock protein (HSP) β-2	Ribeiro et al. (2018)
Trout	Endurance moderate intensity exercise for 4 weeks in a custom circular raceway	107 proteins were differentially abundant	Dindia et al. (2017)
Rat	Endurance exercise by running on a motorized treadmill	Changes in p38 MAP kinase, Myosin light chain kinase 3, Cysteine and glycine-rich protein 3, Bcl2-interacting killer-like protein, and Obscurin	Guo et al. (2017)

40 min/session) in 70–75 years-old male subjects has shown increase in seven proteins including protein z-dependent protease inhibitor, vitronectin, and mannose-receptor 2, which can be associated with thrombosis, cardiovascular disease, and inflammation associated with VO_2 peak/VO_{2max} increase by 10.9% (Hole, 2015). A study of trained and untrained individuals who underwent endurance training has revealed 29 proteins exhibiting significant acute exercise effects in both groups. Seven proteins showed significant differences between the two groups including A2Macro and IL-5 that were elevated in the trained individuals whereas leptin showed higher levels in the untrained group (Schild et al., 2016). Proteomics approach was also utilized for antidoping tests to screen for various recombinant growth hormones (rGH). A study investigating rGH administration for 8 weeks with over a 6-week washout period in non-elite athletes has identified eight plasma proteins associated with rGH (apolipoprotein-L1, alpha-HS-glycoprotein, vitamin D-binding protein, afamin, insulin-like growth factor-binding protein-3, insulin-like growth factor-binding protein-ALS, lumican, and extracellular matrix proteins 1) (Tan et al., 2017; Ohlendieck, 2011).

23.3 Exercise and sports-related metabolomics

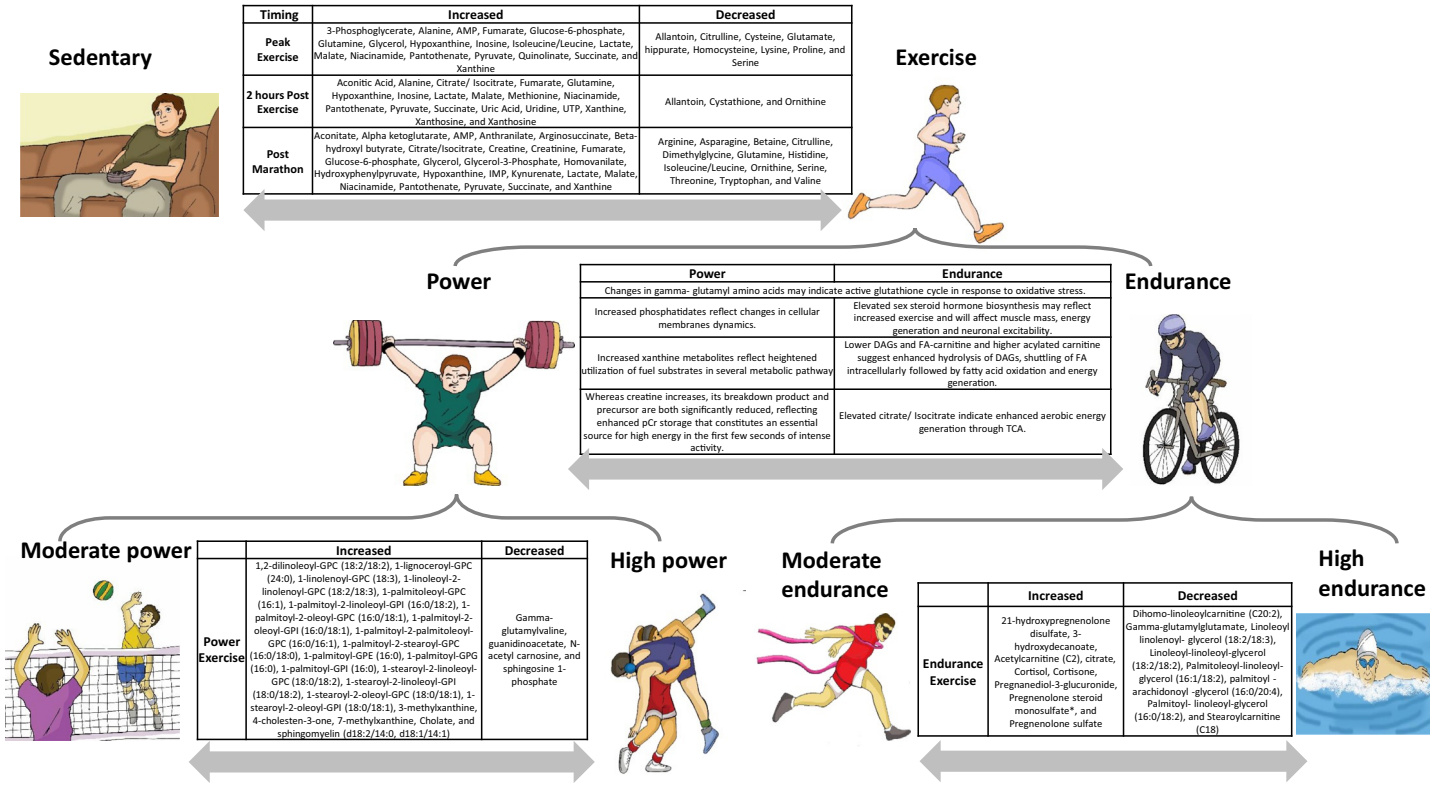
Utilization of metabolomics in research is fast growing. The determination of metabolites levels in a given biological matrix is achieved by certain analytical methods that include untargeted metabolomics, targeted metabolomics, metabolic fingerprinting, metabolic profiling, and exometabolomics (de Raad et al., 2016). Despite significant advances in utilizing NMR-based metabolomics, MS-based metabolomics continues to be the predominant method of choice among metabolomics techniques (Larive et al., 2015; de Raad et al., 2016). The metabolome is made of a wider range of chemicals than the proteome that is covered by 20 amino acids building blocks. The versatility in metabolome's composition is reflected by differences in molecular weights and polarity of metabolites. Furthermore, metabolome covers up to nine order of magnitudes in concentration (from picomolar to millimolar), giving a more comprehensive coverage of diverse compounds that reflect whole body physiology (Dunn and Ellis, 2005). Metabolomics offers a comprehensive tool for identifying metabolic alterations triggered by various environmental factors including diet, exercise and life style in general (Zivkovic and German, 2009). Targeted and non-targeted metabolomics offer a simultaneous analysis of hundreds of metabolites that belong to multiple pathways, providing an accurate phenotype of studied subjects (Nieman and Mitmesser, 2017). Exercise-induced metabolic changes is dependent on the type, intensity, and duration of the training protocols (Lewis et al., 2010; Pitsiladis and Maughan, 1999). Metabolomics permits the identification and quantitation of these changes that reflect various physiological and pathophysiological states of athletes, offering to identify

metabolic signatures that could be used to monitor their health, response to training and performance (Heaney et al., 2017).

Glucose constitutes the major energy fuel in the cell. Circulating levels of blood glucose reflect a balance between food intake and energy consumption. Excess glucose, stored in the liver and skeletal muscle as glycogen, is consumed by intensive exercise to sustain performance (Vandenbogaerde and Hopkins, 2011), causing eventually muscle exhaustion and fatigue. Therefore, glucose levels are used as an indication of athletes' nutritional status and training and exercise workload (Lippi et al., 2008). In response to prolonged exercise that is characteristic of endurance athletes, carbohydrates utilization for energy production becomes exhausted. Consequently, FAs are next utilized as a different energy source. These include circulating medium-chain FAs that get oxidized while entering the liver, leading to energy generation. Some of these also exhibit an anti-inflammatory effect such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which helps in exercise recovery (Bloomer et al., 2009; Jouris et al., 2011; Tartibian et al., 2009) as well as improving neuromuscular function (Stiefel et al., 1999).

Previous studies have identified various exercise-associated metabolites to mark energy metabolism (Yan et al., 2009; Lewis et al., 2010) such as ATP synthesis, FA beta-oxidation and ketone bodies (Heaney et al., 2017). Metabolic differences reflected changes in the consumption of energy substrates in glycolysis (Van Hall et al., 2003), lipolysis (Ormsbee et al., 2007; Goto et al., 2007), adenine nucleotide catabolism (Sahlin et al., 1990a; Muhsen Ali et al., 2016), and amino acid catabolism (Henriksson, 1991) in response to exercise (Chorell et al., 2009; Pohjanen et al., 2007; Lewis et al., 2010). Studies conducted on exercising healthy volunteers has shown a significant reduction in amino acid secretion with enhanced fitness, associated with increased oxidation of FAs (Morris et al., 2013). Metabolomics of athletes' urine after an 800-m race identified metabolic signatures related to TCA cycle, lactic acid cycle, glucose-alanine cycle, hypoxia-induced factors, and oxidative stress (Sun et al., 2017). Physically active subjects who underwent two cycling exercise trials showed changes in a range of purine metabolites and several acyl carnitines. Training for 24 weeks of obese middle-aged men caused functional improvement and metabolic changes in 20 metabolites including tyrosine, 2-oxoisocaproate, histidine, and pyruvate that were identified as the best markers for exercise (Duft et al., 2017). Fig. 23.1A summarizes changes in plasma metabolites at peak endurance exercise, 2-h postexercise and after completion of marathon run as shown by Lewis et al. (2010).

Additionally, metabolomics studies on athletes in response to intensive exercise has shown elevated plasma lactate levels (Goodwin et al., 2007; Berton et al., 2017) as well as breakdown products of adenine (Dudzinska et al., 2010), suggesting active anaerobic metabolism and recycling of ATP. Other reports indicated increased intermediates of TCA cycle in skeletal muscle biopsies, indicating utilization of aerobic energy (Howarth et al., 2004; Peake et al., 2014). Levels of amino acids also changed in response



Timing	Increased	Decreased
Peak Exercise	3-Phosphoglycerate, Alanine, AMP, Fumarate, Glucose-6-phosphate, Glutamine, Glycerol, Hypoxanthine, Inosine, Isoleucine/Leucine, Lactate, Malate, Niacinamide, Pantothenate, Pyruvate, Quinolinate, Succinate, and Xanthine	Allantoin, Citrulline, Cysteine, Glutamate, hippurate, Homocysteine, Lysine, Proline, and Serine
2 hours Post Exercise	Aconitic Acid, Alanine, Citrate/ Isocitrate, Fumarate, Glutamine, Hypoxanthine, Inosine, Lactate, Malate, Methionine, Niacinamide, Pantothenate, Pyruvate, Succinate, Uric Acid, Uridine, UTP, Xanthine, Xanthosine, and Xanthosine	Allantoin, Cystathione, and Ornithine
Post Marathon	Aconitate, Alpha ketoglutarate, AMP, Anthranilate, Arginosuccinate, Beta-hydroxyl butyrate, Citrate/isocitrate, Creatine, Creatinine, Fumarate, Glucose-6-phosphate, Glycerol, Glycerol-3-Phosphate, Homovanilate, Hydroxyphenylpyruvate, Hypoxanthine, IMP, Kynurenate, Lactate, Malate, Niacinamide, Pantothenate, Pyruvate, Succinate, and Xanthine	Arginine, Asparagine, Betaine, Citrulline, Dimethylglycine, Glutamine, Histidine, Isoleucine/Leucine, Ornithine, Serine, Threonine, Tryptophan, and Valine

Power	Endurance
Changes in gamma- glutamyl amino acids may indicate active glutathione cycle in response to oxidative stress.	
Increased phosphatidates reflect changes in cellular membranes dynamics.	Elevated sex steroid hormone biosynthesis may reflect increased exercise and will affect muscle mass, energy generation and neuronal excitability.
Increased xanthine metabolites reflect heightened utilization of fuel substrates in several metabolic pathway	Lower DAGs and FA-carnitine and higher acylated carnitine suggest enhanced hydrolysis of DAGs, shuttling of FA intracellularly followed by fatty acid oxidation and energy generation.
Whereas creatine increases, its breakdown product and precursor are both significantly reduced, reflecting enhanced pCr storage that constitutes an essential source for high energy in the first few seconds of intense activity.	Elevated citrate/ Isocitrate indicate enhanced aerobic energy generation through TCA.

	Increased	Decreased
Power Exercise	1,2-dilinoleoyl-GPC (18:2/18:2), 1-lignoceroyl-GPC (24:0), 1-linolenoyl-GPC (18:3), 1-linoleoyl-2-linolenoyl-GPC (18:2/18:3), 1-palmitoleoyl-GPC (16:1), 1-palmitoyl-2-linoleoyl-GPI (16:0/18:2), 1-palmitoyl-2-oleoyl-GPC (16:0/18:1), 1-palmitoyl-2-oleoyl-GPI (16:0/18:1), 1-palmitoyl-2-palmitoleoyl-GPC (16:0/16:1), 1-palmitoyl-2-stearoyl-GPC (16:0/18:0), 1-palmitoyl-GPE (16:0), 1-palmitoyl-GPG (16:0), 1-palmitoyl-GPI (16:0), 1-stearoyl-2-linoleoyl-GPC (18:0/18:2), 1-stearoyl-2-linoleoyl-GPI (18:0/18:2), 1-stearoyl-2-oleoyl-GPC (18:0/18:1), 1-stearoyl-2-oleoyl-GPI (18:0/18:1), 3-methylxanthine, 4-cholesten-3-one, 7-methylxanthine, Cholate, and sphingomyelin (d18:2/14:0, d18:1/14:1)	Gamma-glutamylvaline, guanidinoacetate, N-acetyl carnosine, and sphingosine 1-phosphate

	Increased	Decreased
Endurance Exercise	21-hydroxypregnenolone disulfate, 3-hydroxydecanoate, Acetylcarnitine (C2), citrate, Cortisol, Cortisone, Pregnanediol-3-glucuronide, Pregnenolone steroid monosulfate*, and Pregnenolone sulfate	Dihomo-linoleoylcarnitine (C20:2), Gamma-glutamylglutamate, Linoleoyl linolenoyl- glycerol (18:2/18:3), Linoleoyl-linoleoyl-glycerol (18:2/18:2), Palmitoleoyl-linoleoyl-glycerol (16:1/18:2), palmitoyl - arachidonoyl -glycerol (16:0/20:4), Palmitoyl- linoleoyl-glycerol (16:0/18:2), and Stearoylcarnitine (C18)

Fig. 23.1 Metabolomic signature of exercise, power, and endurance sports. (A) significantly changing metabolites during peak exercise, 2-h post exercise and after completion of marathon are shown using before exercise as a control (sedentary) (Lewis et al., 2010), (B) metabolic signature of endurance (high and low), and (C) power (high and low) is also indicated as we have previously shown (Al-Khelaifi et al., 2018a).

to high-intensity exercise including increased glutamate in skeletal muscle, causing induction of ammonia metabolism by releasing alanine (Berton et al., 2017; Henriksson, 1991; Leibowitz et al., 2012) and triggering alterations in plasma corresponding metabolites (Sahlin et al., 1990b; Eriksson et al., 1985). Intensive exercise was also shown to induce sex steroid hormones in elite endurance athletes (Sato et al., 2016).

Although various studies have addressed metabolic changes in response to exercise, new research has investigated metabolic changes in various groups of athletes who belong to different sports groups, namely dynamic (endurance) and static (power) sports. This grouping is based on the cardiovascular demand (stroke volume and blood pressure) of athletes who belong to individual sports (Asmussen, 1981; Mitchell et al., 2005). Whereas the former sports are associated with effects on the muscle length due to regular contractions, causing limited but sustainable intramuscular power, the latter causes greater intramuscular power with small changes in muscle length. The two types also differ in their energy requirement. Aerobic energy is mainly utilized in the dynamic exercise whereas anaerobic energy is mostly characteristic of power sports. Table 23.3 defines the types of sports and their characteristics as modified from Mitchell et al. (2005).

Table 23.3 Categorization of dynamic and Static exercise

	Dynamic (Endurance)		**Static (Power)**	
Definition	Changes in the muscle length due to regular contractions producing a limited intramuscular power		A greater intramuscular power with little changes in muscle length	
Subcategorization	The maximal oxygen uptake percentage (VO_2 peak/VO_{2max}) that is achieved with maximum cardiac output		Maximal voluntary contraction percentage (MVC%) gained with increasing blood pressure	
	Moderate (<70% VO_2 peak/VO_{2max})	High (>70% VO_2 peak/VO_{2max})	Moderate (<50% MVC)	High (>50% MVC)
	Aquatics and Athletics	Marathon running, soccer and long-distance triathlons	Canoe sprint (200 m), table tennis, and volleyball	Wrestling, weight lifting, jumping, and throwing
	Some sports, however, require both power and endurance such as cycling, boxing, and rowing.			
Energy	Mostly obtained through oxidative phosphorylation that requires oxygen for the synthesis of ATP through activation of components of the mitochondrial respiratory chain		Largely obtained without the use of oxygen through anaerobic glycolysis where ATP is produced by substrate-level phosphorylation reactions	

23.3.1 Endurance sports-associated metabolic changes

Endurance sports such as long-distance running, rowing, and swimming involve an intense cardiovascular exercise for a long period of time (Joyner and Coyle, 2008), causing chronic adaptations at the cellular and systemic levels (Hackney, 2006; George et al., 2012). Physiological adaptations include alterations in the central regulation of blood flow and oxygen supply, induction of glycogenolysis and gluconeogenesis, lipolysis and FA mobilization, and energy utilization by the skeletal muscle (Hawley et al., 2014). Adaptations also involve the sympathomedullary, adrenomedullary, and the hypothalamus/pituitary adrenal axes, causing changes in the release of various hormones such as the corticotrophin-releasing hormone, arginine vasopressin, adrenocorticotropic hormones, glucocorticoids, and catecholamines (Clark and Mach, 2016). Studies of metabolomics of endurance athletes are still limited. Metabolomics of senior male rowers following 2 weeks training identified changes in levels of hemoglobin, testosterone, and creatine kinase as well as metabolites involved in glucose metabolism, oxidative stress, and energy metabolism such as alanine, lactate, β-D-methylglucopyranoside, pyroglutamic acid, cysteine, glutamic acid, citric acid, free FAs, valine, glutamine, phenylalanine, and tyrosine (Yan et al., 2009). Metabolomics profiling of 20 endurance athletes following two 60-min endurance exercise at a moderate altitude revealed reduction in glucose, glutamine, alanine, and branched-chain amino acids, suggesting that involvement of the protein pathway was necessary but not sufficient for the maintenance of glycemia at moderate altitudes (Messier et al., 2017).

In a large metabolomic profiling of 191 elite athletes including 121 high and 70 moderate endurance athletes, 38 metabolites out of 743 were significantly altered between the two studied groups (Fig. 23.1B) (Al-Khelaifi et al., 2018a). These included a reduction in gamma-glutamyl amino acids in high endurance athletes, indicating an active glutathione cycle for scavenging oxidative stress, and an increase in sex hormone steroids involved in testosterone and progesterone synthesis, suggesting an enhanced endogenous steroid biosynthesis. High endurance athletes also exhibited reduced diacylglycerols (DAGs) and FA-carnintine associated with increased acylated carnitine, indicating an active beta oxidation of FA that involves hydrolysis of DAGs, carrying FA into the cells, followed by their oxidation to generate energy (Hoppel, 1982). This metabolic signature also suggested that exercising muscles of high endurance athletes were more able to activate lipolysis as lipids and FAs become favored substrates as energy resource. The higher levels of acylated carnitine in the high endurance athletes could also suggest a better protection and recovery for exercising muscle as carnitine can lower lactate accumulation (Karlic and Lohninger, 2004). High endurance athletes also showed elevation in some intermediates of TCA cycle such as citrate and isocitrate, suggesting increased utilization of aerobic energy via TCA.

23.3.2 Power sports-associated metabolic changes

Metabolomics studies of power sports are very scarce. Metabolomic profiling of 47 high and 144 moderate power athletes identified 33 differentially expressed metabolites between the two groups (Fig. 23.1C) (Al-Khelaifi et al., 2018a). High-power athletes showed a increase in creatine and a decrease in creatinine (breakdown product of creatine) and guanidinoacetate (precursor of creatine), suggesting active cycle of creatine metabolism (Walker, 1979). Creatine plays an important role in storing and transferring phosphate-bound energy. The active creatine metabolism cycle in high-power athletes suggests a better equipped muscular storage of phosphocreatine for energy generation during exercise by replenishing ATP in the first seconds of intensive activity. Two adenine break-down products (3 and 7 methylxanthine) were also elevated in high power athletes, suggesting elevated utilization of adenine as a source of energy (Tullson and Terjung, 1991). Supplementation of xanthine has been shown to allow athletes to train at higher power output for extended periods (Graham, 2001). High-power athletes also exhibited increased levels of phosphatidates derivatives, suggesting alterations in dynamics of cellular membranes in response to oxidative stress (Powers and Jackson, 2008). Among elevated phosphatidates are the inositol phospholipids that tend to accumulate in hypoxic contracting muscles (Coburn et al., 1988) and 12,13-DHOME long-chain FAs known to stimulate adipogenesis through activating proliferator-activated receptor (PPAR) gamma 2 ligand (Lecka-Czernik et al., 2002). On the other hand, high-power athletes have exhibited lower levels of N-acetylcarnosine, a potent scavenger of lipid peroxidation products by stabilizing the adducts with its imidazolium group (Boldyrev and Abe, 1999). When comparing power and endurance-associated metabolites, common pathways (anti-oxidative stress) are apparent, however unique metabolic pathways are also seen power and endurance (Table 23.4).

When comparing metabolic profiles among individual sports, clear metabolic signatures distinguishing individual sports are revealed. Enrichment analysis has identified FDR significant pathways that are altered between different sports (unpublished data, Fig. 23.2). Most of these differences were identified in lipid metabolites (lyso and phospholipids) that may indicate different cell membrane dynamics and cellular signaling pathways.

23.3.3 Nutrition and supplements-associated metabolic profiling

The nutritional needs of athletes are dependent on their training intensity and sport type. Optimal diet improves performance and recovery from injury and intensive exercise. Compromised muscular function may result from deficiencies in vitamins (such as D and B12), iron, and folate. Hence, maintaining a controlled and balanced diet would ensure avoidance of possible deficiencies-associated with intensive exercise. Hydration

Table 23.4 Power and endurance metabolic signatures and their functional relevance (Al-Khelaifi et al., 2018a)

Power	Endurance
Changes in gamma-glutamyl amino acids may indicate active glutathione cycle in response to oxidative stress	
Increased phosphatidates reflect changes in cellular membranes dynamics	Elevated sex steroid hormone biosynthesis may reflect increased exercise and will affect muscle mass, energy generation, and neuronal excitability
Increased xanthine metabolites reflect heightened utilization of fuel substrates in several metabolic pathway	Lower DAGs and FA-carnitine and higher acylated carnitine suggest enhanced hydrolysis of DAGs, shuttling of FA intracellularly followed by FA oxidation and energy generation
Whereas creatine increases, its breakdown product and precursor are both significantly reduced, reflecting enhanced phospho-creatine storage that constitutes an essential source for high energy in the first few seconds of intense activity	Elevated citrate/Isocitrate indicate enhanced aerobic energy generation through TCA

state of athletes is equally important as water plays a crucial role in regulating the structure and function of cells. A hypohydrated state of body is associated with reduced athletic performance which marks increased risk of heat exhaustion due to plasma water loss (Hoffman et al., 2010). Therefore, careful monitoring of athletes nutrition and hydration state may help in avoiding potential deficiencies with increasing training load. Assessment of nutritional consumption through specific biomarkers may offer a better assessment of athletes metabolic changes and requirements (Beck et al., 2015).

Proteins constitute the building blocks of muscle fibers and can also be utilized as an additional power source for muscles. Measurements of levels of certain proteins in exercising athletes could offer important information about their health and how to improve their training. Albumin levels could indicate performance and recovery from exercise and a decrease in its intake leads to a reduction in its synthesis (James and Hay, 1968). Urea nitrogen levels in urine and circulation are also markers of protein consumption and breakdown as well as hydration status, inflammation, urea synthesis, and excretion. Lower levels of urea nitrogen could reflect a decrease in protein consumption, undernourishment, or liver metabolic problems. Elevated urea nitrogen could be a result of intensive workout, heightened catabolism (Hong and Lien, 1984), or increased protein consumption (Young et al., 2000). Total protein levels are also important indicators of athletes' health. Lower levels of total protein in addition to a decrease in albumin and increase in urea nitrogen could be a sign of inadequate protein consumption or a deterioration in health.

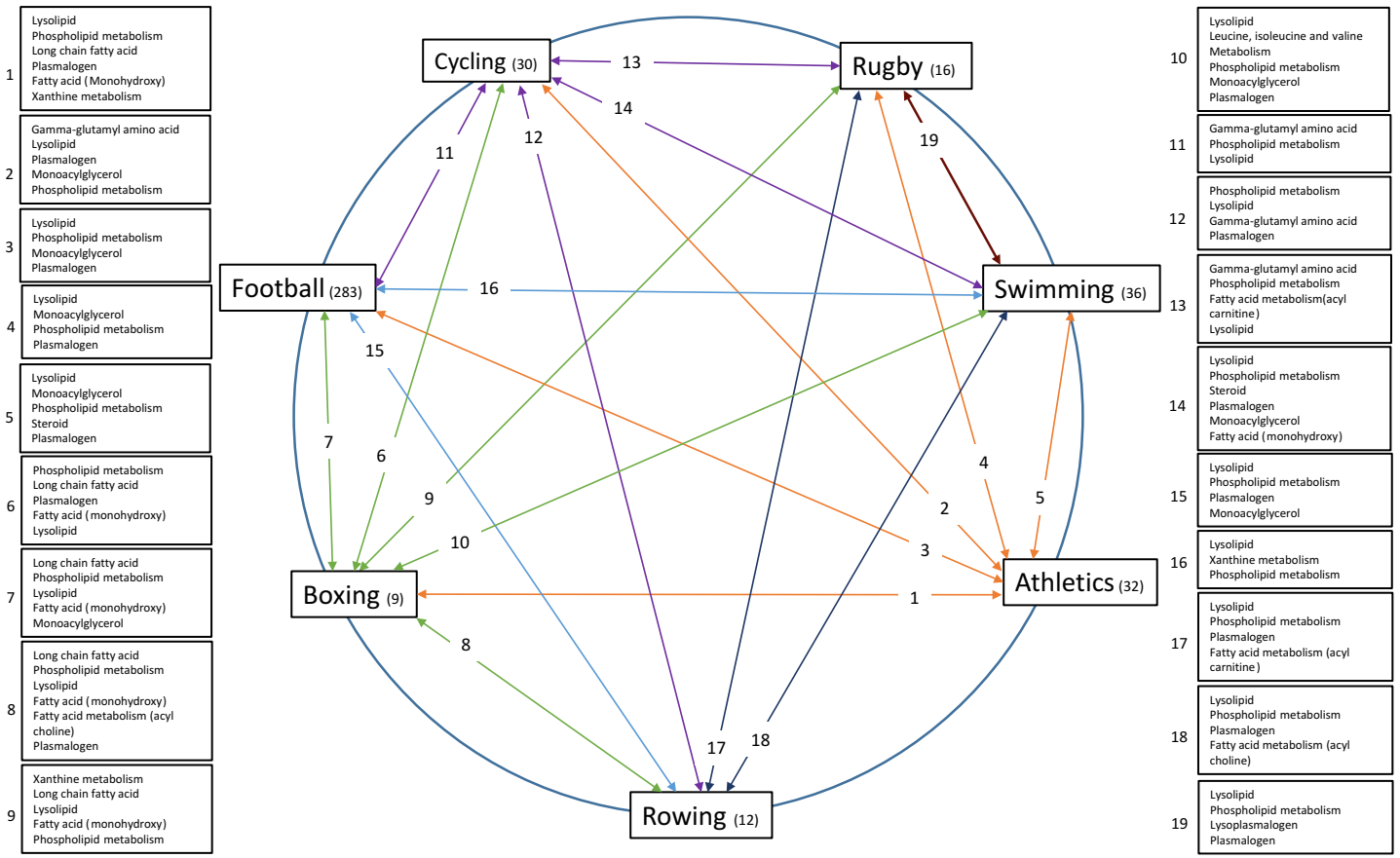


Fig. 23.2 Enriched metabolic pathways differentiating individual sports. Metabolic profiling of serum samples from 418 elite athletes was compared among participants' sport groups (30 cyclists, 283 football players, 9 boxers, 12 rowers, 32 athletics, 36 swimmers, and 16 rugby players). Enrichment analysis was conducted and metabolic pathways that were significantly (FDR significant) altered between the compared groups are indicated in the boxes with numbers indicating the compared groups.

Low carbohydrate and ketogenic diets are popular among athletes. Diets based on 7% carbohydrates are sufficient to maintain performance without influencing gluconeogenesis (Webster et al., 2016) by increasing their FA oxidation to sustain glycogen levels stored in muscles (Volek et al., 2016). Dietary supplements are commonly used by athletes as they offer a better health and performance (Knapik et al., 2016). These include a wide varieties of supplements such as vitamins and minerals, in addition to different types of proteins, creatine, and other ergogenic mixtures (Maughan et al., 2007). Many athletes, however, are unaware of the true benefits or potential risks of consumed supplements because of exaggerated marketing that lacks scientific evidence (Maughan et al., 2007). This is also exacerbated by elite athlete's unwillingness to participate in trials to test the effectiveness of new supplements as a result of their intensive exercise and busy schedules. Therefore, many groups investigated the effects of supplements intake on performance of recreational athletes instead or only looked at information provided by elite athletes in shape of surveys and interviews. A survey for the use of nutritional supplements in 1620 Norwegian elite athletes showed more prevalent use of supplements in male elite athletes than in controls (Sundgot-Borgen et al., 2003). Another study of information provided by 361 Danish elite athletes on doping forms data showed 100% prevalence of use of at least one type of nutritional supplement (Solheim et al., 2017). A third survey of prevalence of dietary supplements in 164 elite young German athletes showed 80% use if at least one type of supplement including minerals and vitamins as well as energy drinks. Carbohydrates are also among the most frequently consumed type of supplements, while few studies reported intake of protein, creatine, and other ergogenic supplements (Braun et al., 2009).

Although metabolomics is widely used in toxicology and pharmacology, the use of metabolomics in human nutrition is still somewhat limited. The measurement of the effect of dietary changes on metabolomics is complex as the metabolic profile is affected by other non-nutritional environmental factors, where interactions are further complicated by the micro flora of the guts, resulting in a complex human nutrition metabolomics (Gibney et al., 2005). Our lab has recently performed profiling of xenobiotics in 478 athletes, aiming at identifying sports group-related dietary supplements (Al-Khelaifi et al., 2018b). The identified xenobiotics included those that prolong exercise tolerance, provide a nootropic effect, exert a potent antioxidant effect or originate from drugs for different types of injuries (Table 23.5).

23.4 Limitations of OMICS in biomarkers discovery

Since performance and health of athletes are complex phenotypes, biomarkers identified by proteomics and metabolomics research may not be specific and sensitive enough as they only represent an instantaneous snapshot of the physiological status of an athlete at a particular point of time (Peng et al., 2015). Furthermore, the exact

Table 23.5 Xenobiotics identified in athletics, footballers and boxers with their potential functional relevance to athletes' health and performance

Sport discipline	Metabolites	Evidence
Athletics	Eugenol	A strong antioxidant present in some plants and herbs. It is also present in certain supplements for purifying blood and reducing risk of cardiovascular risk (Lahlou et al., 2004)
	Stachydrine	Present in a number of citrus fruits and certain supplements that claim reducing stress and anxiety. It has also an osmo-protective role and anti-inflammatory and antioxidant roles (Zhang et al., 2018)
Football	Caffeic acid	Ubiquitously made by various plants such as thyme, sage, spearmint, cinnamon, star anise, black chokeberry, tea, and coffee. It is also present in various supplements. Animals treated with caffeic acid exhibit an enhanced exercise tolerance, reduced lactate and hepatic oxidation (Novaes et al., 2012) with potent antioxidant properties (Olthof et al., 2001, Jung et al., 2006). Caffeic acid phenethyl ester protects from exercise-associated hyperthermal stress (Chen et al., 2009)
	Ferulic acid 4-sulfate	A strong antioxidant that is ubiquitously made by various plants such as wheat, rice, peanuts, oranges, and apples (Graf, 1992)
	Quinine	An essential component of quinate that is used by footballers to treat muscle cramps (Diener et al., 2002)
	Hippurate	Present in various fruits and certain anti-bacterial drugs. Levels are associated with reduced risk of metabolic syndrome (Pallister et al., 2017)
Boxing	Retinol (vitamin A)	A nutritional supplement with strong antioxidant effects, found in anti-aging creams (Kafi et al., 2007)
	2-Pyrrolidinone	A neuroprotective drug with nootropic properties (Shorvon, 2001)
	Thioproline	An antioxidant and free radical scavenger that promotes immune response. It can trigger an anorexic effect in animal model, increasing their survival and improving their neurological functions (Navarro et al., 2007)

Modified from Al-Khelaifi, F., Diboun, I., Donati, F., Botre, F., Alsayrafi, M., Georgakopoulos, C., Yousri, N. A., Suhre, K., Elrayess, M. A. 2018b. Metabolomics profiling of xenobiotics in elite athletes: relevance to supplement consumption. J. Int. Soc. Sports Nutr. 15, 48.

concentrations of specific metabolites might not provide a real measure of performance, therefore investigating differences in levels of these metabolites over periods of intensive exercise would provide a more accurate estimation of athletic performance and ways for better adaptation to exercise and improved recovery. The variability in reference ranges of potential biomarkers in athletes from different sports also adds to the complexity of identifying a suitable biomarker (Lewis et al., 2016). Therefore, a more comprehensive representation of athletes' nutrition, hydration, metabolic health, energy utilization, muscle status, fatigue, inflammation, and injury risk could collectively present a more accurate view of an athlete's metabolic status and general health in response to their unique diet and exercise. This can be achieved by measuring biomarkers not only during intensive training seasons but also during off-seasons when an athlete is rested and healthy to gain a better prospective of their dynamic ranges (Krug et al., 2012). Implementation of biomarker monitoring in sport assessment and coaching would require a cautious selection of candidate biomarkers, a precise testing schedule, and an informed interpretation of data with respect to physiological and pathophysiological status of an athlete.

23.5 Conclusions

Advancement in OMICS platforms has provided a comprehensive and deep phenotyping of athletes' metabolic pathways that vary according to their exercise intensity and duration, sport type, and level of fitness. Proteomics and metabolomics research has revealed a number of functional proteins and metabolic pathways altered with exercise or between different sports. Changes were mostly detected in the cytoskeletal structure or signaling pathways marking mitochondrial oxidative mechanisms and other energy-related pathways. A number of potential biomarkers differentiating exercising skeletal and cardiac muscles was identified but requires further validation before functional application. Metabolomics of professional athletes dichotomized according to their sport type-specific cardiovascular demands into endurance or power has revealed specific metabolic signatures associated with steroids biosynthesis and glutathione metabolism, in addition to energy substrates marking glycolysis, lipolysis, adenine nucleotide, and amino acid catabolism. These signatures may serve as potential biomarkers of exhaustive exercise of professional athletes for improved training outcome, prevention of exhaustion-associated injuries, and better health and performance in general. Alterations in these metabolic pathways could also offer invaluable tools for antidoping research that is related to athletes biological passport in conjugation with other tests or as a stand-alone test. Athletes from different sporting disciplines follow different dietary protocols that include various nutritional supplements to improve their health and boost their performance. These supplements include xenobiotics known to improve exercise tolerance, produce a nootropic effect, show a strong antioxidant properties or constitute an ingredient of various drugs targeting exercise-related injuries. Utilization of OMICS research in sports science

is still at its infancy and suffers from certain limitations related to the natural variation of metabolites ranges within an individual and among individuals. Therefore, more research is needed to confirm these potential biomarkers in different sports groups at various time points in order to provide consistent and more informative metabolic biomarkers of athletic health and performance.

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Index

Note: Page numbers followed by *f* indicate figures and *t* indicate tables.

B

C

D

E

F

G

J

K

L

N

O

P

Q

R

S

T

U

V

W

X

Y

Z

Sports, Exercise, and Nutritional Genomics

Current Status and Future Directions

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Sports, Exercise, and Nutritional Genomics: Current Status and Future Directions is the first reference volume to offer a holistic examination of omics-driven advances across different aspects of exercise and sports physiology, biochemistry, sports medicine, psychology, anthropology, and sports nutrition; and highlighting the opportunities towards advance personalized training and athlete health management. More than 70 international experts from 14 countries have discussed key exercise and sport-related themes through the prism of genomics, epigenomics, transcriptomics, proteomics, metabolomics, telomere biology, talent in sport, individual differences in response to regular physical activity, that in the future may empower coaches, sports physicians, fitness experts, genetic counselors, and translational scientists to employ various omics data and approaches in improving health and physical performance of people participating in sports and exercise activities.

Key Features

- Effectively synthesizes key themes across molecular aspects of exercise and sports sciences.
- Provides a knowledge base for future translation of omics solutions to talent identification, individualized training, and nutrition.
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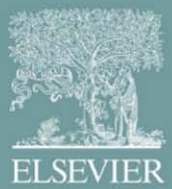

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