Scand J Med Sci Sports 2016: 26: 600–612
doi: 10.1111/sms.12503

SCANDINAVIAN JOURNAL OF MEDICINE & SCIENCE IN SPORTS

Review Article

The heritable path of human physical performance: from single polymorphisms to the "next generation"

C. G. M. Santos[1,2], P. M. Pimentel-Coelho[1,2], B. Budowle[3,4], R. S. de Moura-Neto[5], M. Dornelas-Ribeiro[1], F. A. M. S. Pompeu[6], R. Silva[2]

[1]Instituto de Biologia do Exército, Brazillian Army Biologic Institute, Rio de Janeiro, Brazil, [2]Instituto de Biofísica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil, [3]Molecular and Medical Genetics, University of North Texas – Health and Science Center, Fort Worth, Texas, USA, [4]Center of Excellence in Genomic Medicine Research (CEGMR), King Abdulaziz University, Jeddah, Saudi Arabia, [5]Instituto de Biologia, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil, [6]Escola de Educação Física e Desportos, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil
Corresponding author: Caleb Guedes Miranda dos Santos, Instituto de Biologia do Exército, Brazillian Army Biologic institute, Rua Francisco Manuel 102, Benfica, Rio de Janeiro, RJ 20911-270, Brazil. Tel: +55 21 3890-2135, Fax: +55 21 3890-2146, E-mail: calebguedes@ibest.com.br

Accepted for publication 12 May 2015

Human physical performance is a complex multifactorial trait. Historically, environmental factors (e.g., diet, training) alone have been unable to explain the basis of all prominent phenotypes for physical performance. Therefore, there has been an interest in the study of the contribution of genetic factors to the development of these phenotypes. Support for a genetic component is found with studies that shown that monozygotic twins were more similar than were dizygotic twins for many physiological traits. The evolution of molecular techniques and the ability to scan the entire human genome enabled association of several genetic polymorphisms with performance. However, some biases related to the selection of cohorts and inadequate definition of the study variables have complicated the already difficult task of studying such a large and polymorphic genome, often resulting in inconsistent results about the influence of candidate genes. This review aims to provide a critical overview of heritable genetic aspects. Novel molecular technologies, such as next-generation sequencing, are discussed and how they can contribute to improving understanding of the molecular basis for athletic performance. It is important to ensure that the large amount of data that can be generated using these tools will be used effectively by ensuring well-designed studies.

Human physical performance is a complex multifactorial trait that is affected by environmental factors such as diet and training, as well as hereditary factors (Tsianos et al., 2010; Eynon et al., 2011d). Environmental factors alone are unable to characterize all prominent phenotypes for human physical performance, which has resulted in greater interest in the study of the contribution of genetic factors contributing to these traits (Myburgh, 2003).

Initial studies on physical performance aimed to indirectly evaluate the genetic basis of human physical performance through heritability estimates attributable to genes, using variability in phenotypic traits of individuals in family studies, especially those comparing such traits in monozygotic and dizygotic twins (Komi et al., 1973; Fagard et al., 1991; Sundet et al., 1994; Bouchard et al., 1997; Calvo et al., 2002; Mangino, 2014). For instance, the genomic component may account for up to 90% of the variance in maximal oxygen uptake (VO_{2max}) and the proportion of different types of muscle fibers in twins (Bouchard, 2012; Pitsiladis et al., 2013). Focusing on sex-related differences, other studies have shown interindividual variability in muscular response to controlled resistance training (Erskine et al., 2010); while relative strength gains are higher in women, increases in absolute and relative muscle size are slightly higher in men (Hubal et al., 2005; Pescatello et al., 2013).

It is estimated that between 20% and 80% of a wide variety of traits relevant to physical performance, including oxygen uptake, cardiac output, relative proportion of fast and slow fibers, and muscle strength phenotypes (cross-section area and isometric, concentric, and eccentric strength) are highly influenced by genetic factors (MacArthur & North, 2007; Moor et al., 2007; Bouchard, 2012). Moreover, even the predisposition for adherence to sports and the competitive level achieved by individuals may be influenced by genetics (Stubbe et al., 2006; Moor et al., 2007; Mustelin et al., 2011; Costa et al., 2012). However, various studies in recent years have failed to elucidate the associations between specific genotypes and athletic ability (Amir et al., 2007; Dias, 2011; Pitsiladis et al., 2013).

Clearly, physical performance is determined by various biological and environmental factors, and, therefore it is unlikely that a single gene will determine the "athlete" phenotype. Studies on the association between genetics and physical activity revealed several loci that may potentially influence aerobic and anaerobic energy parameters, and an increasing number of polymorphisms associated with sports competence phenotypes are reported every year. In fact, over 200 genes associated with physical performance already have been described, with some of them related to elite sports performance (Rankinen et al., 2006; Bray et al., 2009; Santiago et al., 2010; Eynon et al., 2011c).

It should be noted that the goal of such studies is not to ascertain the genetic determination of a single individual phenotype, but instead seek to identify several factors that jointly determine the final phenotype. For instance, factors such as mitochondrial density, oxidative enzyme activity of myocytes, composition of muscle fibers, capillarization of skeletal muscle and myocardium, VO_{2max}, aerobic capacity, and cardiovascular efficiency may, in combination with other factors, result in a favorable phenotype for a long-distance runner (Ahmetov et al., 2007; Ben-Zaken et al., 2015).

Despite the increasing number of candidate genes and the possibility of finding a "more favorable" polygenic profile in elite athletes of a given sport (Ruiz et al., 2009; Eynon et al., 2011c), the results of these studies often are conflicting or difficult to extrapolate to actual performance. The factors that render comparisons between these studies problematic include insufficient comparative analyses across genders, ethnicities, and sport fields, the different types of parameters measured for a given individual trait, differences in the experimental design, and sample size (Zilberman-schapira et al., 2012). Moreover, most studies reported data on a single polymorphism, which limit the ability to understand individual variations in human athletic performance, as a single gene may contribute only a small portion of the heritable component (Santiago et al., 2010).

Further studies are needed to fully understand the discrete role of genetic factors in athletic performance and ability in different sports and specialties (Henderson et al., 2005). For instance, success in endurance sports, which requires increased physical strength, depends on a high level of aerobic or cardiorespiratory fitness that is often represented by VO_{2max} and resistance levels (Bouchard et al., 1998). Conversely, explosive power athletes rely on anaerobic activity and muscle speed. These two main sport fields involve different types of muscle metabolism. Sprint and power events require predominantly anaerobic or high-energy production muscle metabolism. Thus, it is unusual to find an athlete who excels in both 100 m and 10 000 m races, i.e., elite sprinters are likely not to perform well in long-distance events and vice versa (Tesch & Karlsson, 1985; Amir et al., 2007).

The aim of this review is to provide an informative overview covering the important data about heritable genetic aspects of athletic performance. A discussion is included on how next-generation sequencing can be used to improve the knowledge of the genetic contribution to athlete performance.

Genetic polymorphisms associated with physical performance

Genetic polymorphism is the existence of multiple, or variant, alleles at a locus and the variant occurs at a frequency greater than 1% in the general population (Lewin, 2009). Several polymorphisms have been associated with physical performance, but none have been as widely investigated as the angiotensin-converting enzyme (*ACE*) and alpha-actinin 3 (*ACTN3*) genes (Ahmetov et al., 2013). The polymorphism of an insertion (I) or deletion (D) of 287 base pairs in intron 16 of the *ACE* gene was the first to be associated with physical performance. The I allele (insertion) may be associated with lower plasma and tissue *ACE* levels, better energy balance in long-term exercise, and increased performance in endurance activities in Caucasians (Rigat et al., 1990; Santiago et al., 2010; Holdys et al., 2011; Ma et al., 2013).

In contrast, the D allele, related to higher ACE activity, was initially associated with sprint-power performance (Cerit et al., 2006). Given that angiotensin II can act as a growth factor through the AT1 receptor pathway (Gordon et al., 2001), it would be natural to expect a greater gain in muscular strength and hypertrophy in individuals with this allele. However, there are conflicting results involving the response of individuals with the D allele to training (Semprun-Prieto et al., 2011). In addition, several studies showed that the infusion of angiotensin II can induce atrophy of skeletal muscle tissue (Tabony et al., 2011; Rezk et al., 2012) and recent investigations have observed only a milder association between the ACE polymorphism and muscle volume growth after controlled training (Charbonneau et al., 2008; Erskine et al., 2014). Conflicting results have been reported in the field of high-performance sports, especially in populations that traditionally reveal world-class athletes, such as Ethiopians, Kenyans (for endurance) and Jamaicans (sprinters – runners), whose performances were not associated with *ACE* polymorphisms (Scott et al., 2005a; Ash et al., 2011; Holdys et al., 2011).

The human *ACTN3* gene encodes alpha-actinin-3, which is a sarcomeric actin-binding protein that plays a fundamental role in the maintenance and regulation of the cytoskeleton. Alpha-actinin-2 and alpha-actinin-3 are structural proteins in the myofibril Z-line that form a crystalline structure that anchors along the thin filaments containing actin. They stabilize the contractile apparatus and are important for muscle contractions (McCauley

et al., 2008; Norman et al., 2009; Schiaffino & Reggiani, 2011; Ma et al., 2013). Gene variants or polymorphisms can alter the function of a protein. Single nucleotide polymorphisms (SNPs) are the most common type of genetic polymorphism and refer to a single nucleotide change (adenine-A, thymine-T, cytosine-C, and guanine-G) in the genomic DNA at a specific position in the genome that is observed between members of the same biological species or chromosomes among individuals. The most common *ACTN3* SNP generates a stop codon in the gene, resulting in premature termination of protein synthesis in the tissue. The portion of the population worldwide containing the genotype of two deficient alleles (XX) is approximately 1 billion people. These people are completely deficient in alpha-actinin-3 (Hong & Jin, 2013). The function usually performed by *ACTN3* is possibly performed by other proteins such as alpha-actinin 2. However, the latter is found in all human skeletal muscle fibers (I, IIA, and IIx), whereas expression of alpha-actinin-3 (R allele) in muscle is restricted to fast fibers (IIA and IIx), which are more prevalent among sprint athletes (Berman & North, 2010; Lek et al., 2010; Broos et al., 2012; Grealy et al., 2013). Thus, it is possible that alpha-actinin-2 may not completely compensate for the lack of alpha-actinin-3, and therefore, polymorphisms in the *ACTN3* gene may possibly be associated with elite athletic performance, in sprint (R allele) or endurance (X allele) activities (Eynon et al., 2012; Mikami et al., 2014). However, results are conflicting when different ethnic populations are considered (Yang et al., 2007; Alfred et al., 2011; Cięszczyk et al., 2011; Eynon et al., 2013; Pitsiladis et al., 2013), justifying further investigation.

The mechanism of association between the *ACTN3* polymorphism and sprint performance also may be related to muscle fiber type ratio or to the adaptive response to training (Ahmetov et al., 2011; Seto et al., 2013). Young men (21.7 ± 2.3 years) with the "RR" genotype, which indicates the presence of functional *ACTN3* in its two alleles, showed higher relative torque in dynamic knee extension exercise ($45–300°s^{-1}$) than individuals with the "XX" genotype. Additionally, "RR" individuals had a higher proportion of fast-twitch type IIx fibers than "XX" individuals (Vincent et al., 2007). However, a similar study found increased torque but no differences in fiber types (Norman et al., 2009). Testing of skinned single fibers from the quadriceps muscle of three men with spinal cord injury, who each carried one of three possible genotypes ("RR," "RX," and "XX"), showed that the "XX" carrier's biopsy was the only one that presented slow type I fibers (typical of endurance athletes) with a complete lack of type IIx fibers, and the preservation of type IIa fibers (Broos et al., 2012). The influence of the polymorphism in the *ACTN3* gene also has been supported by studies in 129X1/SvJ mice, in which muscle from *ACTN3* knock-out mice displayed reduced strength, muscle mass, lean mass, fast fiber diameter, and altered contractile properties compared with wild-type mice, except in the soleus muscle, which is rich in slow-twitch type I fibers (Macarthur et al., 2008; Berman & North, 2010). Recent findings in C57BL/6J and C57BL/10ScSn mice showed that alpha-actinin-2 expression in place of alpha-actinin-3 (XX genotype) may increase the availability of calcineurin, an important signaling protein strongly associated with muscle hypertrophy and remodeling in response to exercise. This change would lead to the expression of a fiber profile characterized by slower contraction and by an oxidative phenotype, which is important for endurance performance (Jiang et al., 2010; He et al., 2011; Seto et al., 2013). Nevertheless, the differentiation of muscle fibers is a multifactorial phenotype, and several other polymorphisms have been associated with such differentiation such as the polymorphisms in the *ACE*, *HIF1A*, *PPARs*, *UCPs*, and *VEGFA* genes (Ahmetov et al., 2012; Maciejewska-Karlowska et al., 2014).

Several other polymorphisms that presumably influence physiology have been classically associated with elite physical performance (Fig. 1). These polymorphisms include genes associated with (1) muscle structure and development such as myostatin (*MSTN* or *GDF-8*), muscle creatine kinase (CK-MM), and *ACTN3* (Zhou et al., 2006; Martínez et al., 2009; Norman et al., 2009; Santiago et al., 2011); (2) general metabolism and mitochondrial function/oxidative phosphorylation such as uncoupling proteins 2 and 3 (*UCP2* and *UCP3*) and peroxisome proliferator-activated receptors (*PPARA*, *PPARGC1A*, and *PPARD*; Ahmetov et al., 2009; Ginevičienė et al., 2011; Maciejewska et al., 2011, 2012); (3) blood vessel physiology such as endothelial nitric oxide synthase, hypoxia-inducible transcription factor, and vascular endothelial growth factor (Artioli et al., 2007; Ahmetov et al., 2008, 2009; Bray et al., 2009; Eynon et al., 2010a,b; Serrano et al., 2010); and (4) other physiological pathways such as adenosine monophosphate desaminase 1, angiotensinogen, and bradykinin receptor β2 (Williams et al., 2004; Rubio et al., 2005; de Groote et al., 2006; Lucia et al., 2006; Saunders et al., 2006; Amir et al., 2007).

Despite these most extensively studied polymorphisms, and many others that have been related to performance (Table 1), controversial and nonreproducible results are common and point to observational studies with low statistical power and significance that are hampered by the low frequency of the polymorphism in the study population, the low influence of the polymorphism in the function of its gene, and the low relevance of the polymorphism to the phenotype studied (e.g., VO_{2max}; Bouchard et al., 2011b; Bouchard, 2012; Hong & Park, 2012; Pitsiladis et al., 2013; Yazdi & Robin, 2013). Furthermore, other factors may confound the understanding of the molecular basis of sports performance, such as cognitive, motivational, and social aspects. For example, it has been shown that 33% of the international level

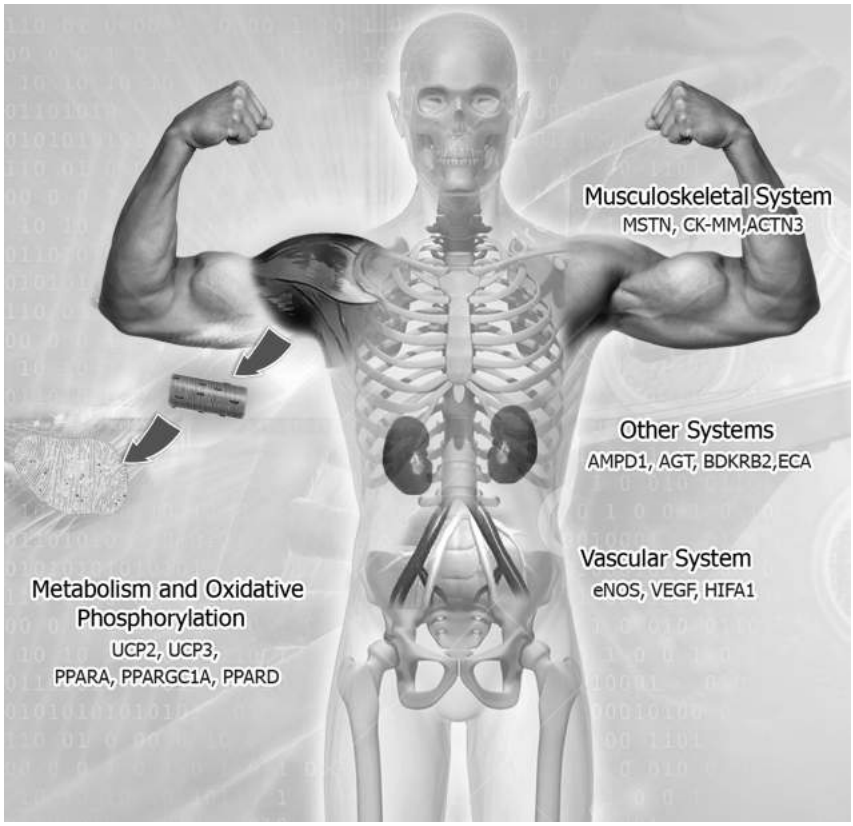

Fig. 1. Some of genetic polymorphisms classically associated with physical performance. MSTN (GDF-8), myostatin; CK-MM, muscle creatine kinase; ACTN3, alpha-actinin-3; UCP2 and UCP3, uncoupling proteins 2 and 3; PPARA, PPARGC1A, and PPARD, peroxisome proliferator-activated receptors; eNOS, endothelial nitric oxide synthase; HIFA-1, hypoxia-inducible transcription factor; VEGF, vascular endothelial growth factor; AMPD1, adenosine monophosphate desaminase 1; AGT, angiotensinogen; BDKRB2, bradykinin receptor β2; ACE, angiotensin converting enzyme.

Kenyan runners indicated that economic empowerment was the main motivation for running, whereas 18% chose tradition as the main motivator (Onywera et al., 2006). The use of drugs and other substances that can drastically influence an athlete's physiology (doping) also is a confounding factor when investigating the contribution of genetic factors on sports performance.

Thus, increasing sample size in association studies is crucial to understanding the relationship between heredity and sports performance (Hagberg et al., 2011). However, obtaining larger samples is complicated by the small number of elite athletes from the same ethnicity in a given sport (Buxens et al., 2010; Eynon et al., 2011b, 2013). Moreover, the low number of publications with negative results complicates the understanding of the actual effect of these polymorphisms in different populations. Positive findings, showing the association of a polymorphism with a given phenotype, are more frequently published than negative results, resulting in a strong publication bias.

In recent years, several advances involving "genomic high throughput technologies" allowed the study of the involvement of a huge number of genes and their polymorphisms in multifactorial complex traits. These technologies have enabled complete sequences of individual human genomes and genome-wide association studies (GWAS) to be applied to the study of sports practice and performance (Bouchard, 2011; Pérusse et al., 2013). Instead of focusing on extremely limited regions of the genome as simple association studies, GWAS investigate associations between phenotypes of interest and genetic variability in a more unbiased manner throughout the genome because the study is not as limited by investigating only hypothesis-driven candidate genes (Kim et al., 2011; Pitsiladis et al., 2013). GWAS do include the screening of candidate genes, whose results must be replicated and confirmed in other populations to be considered strong evidence of positive genetic association (Manolio, 2010; Mangino, 2014). In addition, these screenings may offer a wide range of possibilities for further investigations, including genotype–phenotype studies in exercise physiology. For instance, a very well-controlled study (HERITAGE Family Study) with sedentary Caucasians (483 individuals from 99 families) and African-Americans (259 individuals from 105 families) using a multivariate regression model identified 21 SNPs (Table 1) related to VO_{2max} levels, of which 16 accounted individually for 1.0–7.0% and collectively for 45% of the total variance in VO_{2max} in response to training (Bouchard, 2011). More comprehensive studies are

Table 1. A brief overview of the diversity of physical performance measures used in some studies on genetic polymorphisms published over the last 9 years

	Performance measure	Sample population	Associated markers	Nonassociated markers	Reference
Genotype-phenotype	VO_{2max} (cycle ergometer)	686 patients (stable congestive heart failure)	–	1 (AMPD1)	de Groote et al., 2006
Case Control	Sport ranking Elite classification	121 elite athletes 247 controls	1 (*ACE*)	–	Amir et al., 2007
Genotype-phenotype	Torque–velocity relationship, Knee extensor isometric strength	79 recreationally active	–	2 (*ACE, ACTN3*)	McCauley et al., 2008
Case Control	Sport ranking Elite classification	230 rowers 855 controls	5 (*ACE, ACTN3, NOS3(5/4), UCP2, UCP3*)	–	Ahmetov et al., 2008
Genotype-phenotype	Wingate test, isokinetic exercise, muscle fiber typing, mRNA levels	120 recreationally active	1 (*ACTN3*)	–	Norman et al., 2009
Case Control	Sport ranking, elite classification (power/sprint), TGS	46 endurance athletes 123 controls	7 (TGS of the following genes: *ACE, ACTN3, AMPD, CKMM, HFE, GDF-8, PPARGC1A*)	–	Ruiz et al., 2009
Case Control	Sport ranking Elite classification	153 athletes (100 endurance/53 power) 100 controls	3 (*IL6, NAT2, eNOS-786*)	33 (*ACE, ACTN3, ADRB1, ADRB2(2), ADRB3, AGT, APOA1, APOB(3), APOE(2), GNB3, GSTP1(2), GSTT1, GSTM1, NAT2(6 others), OGG1, DSG2(5), eNOS-894, NPY, SOD2*,)	Buxens et al., 2010
Case Control	Sport ranking Elite classification TGS	153 athletes (100 endurance/53 power) 100 controls	6 (TGS of the following genes: *ACE, ACTN3, AMPD, CKMM, HFE, GDF-8, PPARGC1A*)	–	Ruiz et al., 2010
GWAS/longitudinal	VO_{2max} gain	834 sedentary subjects	21 (*PRDM1, GRIN3A, KCNH8, C9orf27, ACSL1, ZIC4, CAMTA1, RGS18, BIRC7, DBX1, DAAM1, NDN, CXCR5, TTC6, LOC400950, LOC100289626, LOC100130460, NLGN1, MN1, CD44, ENPP3*)	~ 320 000 other SNPs	Bouchard et al., 2011b
Genotype-phenotype	Delta efficiency	85 subjects	2 (*UCP2, UCP3*)	–	Dhamrait et al., 2012
Case Control	Sport ranking Elite classification (power/sprint)	633 elite athletes/808 controls, Spanish/Polish/Russian cohort	1 (*ACTN3*)	–	Eynon et al., 2012
Genotype-phenotype	Maximum isometric patellar tendon force, muscle volume, maximum power, 1-RM	51 untrained subjects	2 (*ACTN3, ACE**)	–	Erskine et al., 2014
Case Control	Sport ranking Elite classification (power/sprint)	299 athletes 649 controls	1 (*ACTN3*)	–	Mikami et al., 2014
Case Control	Sport ranking Elite classification(power/sprint/strength/ endurance and mixed classifications)	660 athletes 704 controls	3 (*PPARD* rs2267668†, rs2016520 and rs1053049) and Haplotypes	–	Maciejewska-Karlowska et al., 2014

*Only combined with *ACTN3*.

†Combined with rs2016520 and rs1053049.

1-RM, one repetition maximum; GWAS, genome-wide association study; SNP, single nucleotide polymorphism; TGS, total genotype score; VO_{2max}, maximal oxygen uptake.

needed with these genes for confirmation of functional allelic variants and to elucidate the mechanisms associated with this possible effect. However, despite the great advances in this field, the GWAS methodology should be applied rigorously in larger cohorts, in which the phenotype of interest is better defined, to circumvent the problem of low statistical power (Manolio, 2010; Kim et al., 2011; Pitsiladis & Wang, 2011; Zhang et al., 2011). Thus, the analysis of larger cohorts, although difficult in the science of elite sports performance, may involve the formation of consortia that combine multiple studies and acquire samples from an adequate number of individuals from different populations, similarly to what has been done in genomic studies from other health science areas, such as diabetes and cardiovascular disease. However, an exaggerated increase of the sample size can strongly favor the appearance of type I statistical error (Jannot et al., 2015) and potentially an incorrect selection of candidate genes. The replication of novel results in different population samples may be more important than the indiscriminate increase of sample size.

The role of mitochondrial inheritance

Mitochondria play a prominent role in physical performance because they generate the essential oxidative capacity in skeletal muscle for endurance activities (Niemi & Majamaa, 2005). VO_{2max}, which is the "gold standard" for aerobic capacity, depends not only on cardiac output, but mainly on the ability of muscles to perform oxidative phosphorylation. In fact, there is a predominance of type I muscle fibers with high density of mitochondria and aerobic metabolism capacity in top endurance athletes who typically have high VO_{2max} values (Eynon et al., 2011c; Mikami et al., 2011).

Several oxidative phosphorylation proteins are encoded by the mitochondrial genome (mtDNA) itself, thus making it an excellent candidate for influence on aerobic performance (Scott et al., 2008; Nogales-Gadea et al., 2011). Although mtDNA genome is small (16569-bp) and contains only 13 protein-coding genes, its analysis can be useful for comparisons among ethnic groups (Eynon et al., 2011b; Mikami et al., 2011). Because mtDNA has a relatively higher mutation rate than the nuclear genome, the genome is maternally inherited without recombination, and the influence of ethnicity on elite athlete performance cannot be overlooked (Calvo et al., 2002; Buxens et al., 2010), mtDNA can be used to examine detailed phylogenies and explore the matrilineal kinship of individuals and populations (Larsson & Oldfors, 2001; Deason et al., 2012; Nunnari & Suomalainen, 2012; Wilber & Pitsiladis, 2012).

Mitochondrial DNA haplogroups, which are groups of similar haplotypes based on polymorphisms that share a common ancestor, reflect the concentration and spatial distribution of matrilineal lineages, which were established through generations during the evolution and spread of humans. In fact, aerobic capacity has been found to have stronger maternal than paternal inheritance, which supports the importance of mitochondria with endurance activities (Nogales-Gadea et al., 2011).

Some studies have associated different mtDNA haplogroups with elite sports performance. Interestingly, haplogroup distribution of Ethiopian endurance runners did not differ from that of the general Ethiopian population (Scott et al., 2005b), whereas international Kenyan athletes differed in their mtDNA haplogroup distribution relative to the general Kenyan population and displayed an excess of L0 haplogroups and a dearth of L3* haplogroups (Scott et al., 2008). Conversely, no significant difference in total haplogroup frequencies was found between Jamaican sprinters and the general population (Deason et al., 2012). Thus, more comprehensive studies are needed because, besides genomic DNA, certain mtDNA haplogroups, subhaplogroups, and polymorphisms may influence human physical performance in a given population (Scott & Pitsiladis, 2007; Maruszak et al., 2014).

Because mitochondrial metabolism is fundamentally different between endurance and sprint/power athletes (Flueck & Eilers, 2010; Eynon et al., 2011a,c), there is great interest in investigating mitochondrial genetic diversity and its complex interactions with nuclear genome, in association with human physical performance, to uncover possible mitochondrial profiles for each type of sports activity.

The role of ethnicity in genetic inheritance

In addition to mitochondrial inheritance, other associations between genetic profile and physical performance seem to occur consistently in certain populations. However, these associations cannot always be extrapolated to other ethnic groups. For instance, the *ACE* polymorphism (intron 16) has been estimated to explain up to 47% of the variance in *ACE* levels in Caucasians, in whom the I allele has been associated with lower *ACE* plasma levels and high endurance performance. Conversely, no differences in *ACE* levels and allelic frequency were observed between Kenyan athletes and the general Kenyan population, indicating that other factors may influence endurance performance in this population (Scott et al., 2005a; Thorn et al., 2010). Also, similar frequencies of the *ACTN3* polymorphism were observed between individuals from Nigeria and Kenya, whose athletes are known to excel in sprint and endurance events, respectively (Yang et al., 2007). These data indicate that the influence of *ACTN3* may be secondary to other genetic and environmental characteristics that differ between populations (Scott et al., 2010). The differences between populations can be striking, with frequencies of the *ACTN3* X allele close to 10% in African and 50% in Eurasian populations (North et al., 1999; North, 2008).

Ethnic differences may be even more complex. In some cases, different biogeographical origins of a given population should be considered. For instance, elite Kenyan endurance runners differed greatly in their biogeographical origin and tribe relative to the general Kenyan population (Onywera et al., 2006). Thus, studying highly heterogeneous and/or admixed populations represents a great challenge to understanding genomics within and among ethnic groups. If the "genetic advantage" is present in an ethnic group, it will not be revealed by comparing cases and controls in the same group, but rather by comparing individuals from different origins. In conclusion, ethnicity is an important variable to consider when examining the role of heredity in physical performance. The conflicting results of some studies may be attributed to the heterogeneity of the populations or the selection of a control group that is not representative of the actual population or that may even be incompatible with the case characteristics (Amir et al., 2007; Zoossmann-Diskin, 2008).

The role of epigenetics in performance

Studies in the post-genomics era have been investigating the variability of gene sequences among individuals and also are focusing more so on the great variability in the form and extent to which each gene is expressed in the absence of changes in DNA sequence. The study of the mechanisms that alter gene expression is called epigenetics (McGee & Hargreaves, 2011; Costa et al., 2012; Raleigh, 2012; Denham et al., 2014). Because they do not alter the nucleotide sequence, epigenetic mechanisms can be a link between environmental stimuli and the regulation of genome function through the activation and inactivation of genes (Sharp, 2010). The variety of mechanisms that regulate gene expression can have a significant impact on physiological/metabolic pathways, particularly if major environmental factors are involved, as occurs in high-intensity training, which can cause efficient adaptation of a phenotype and influence sports performance (Gomez-Pinilla et al., 2011; Ehlert et al., 2013).

Physical exercise is known to induce significant transcriptional changes in peripheral blood cells, skeletal and cardiac muscle, brain, adipose tissue, and buccal cells in humans (Kaliman et al., 2011; Denham et al., 2014). In this regard, the RNA signature of a given tissue might better reflect the complex physiological alterations that occur in response to physical exercise (Bouchard et al., 2011a). An interesting study has observed that, from ~ 800 gene transcripts that were regulated after a 6-week fully supervised endurance training program in 24 young sedentary healthy men, at least 100 genes were differentially regulated between the highest and the lowest responders for aerobic capacity (Keller et al., 2011).

The major mechanisms by which the gene responds to environmental variation include covalent modifications of DNA and histones such as methylation, as well as the presence of transcription factors, coactivators, and noncoding RNAs, including long noncoding RNA (lncRNA) and microRNAs (miRNA; Sanchis-Gomar et al., 2012; Konopka & Sreekumaran Nair, 2013). For instance, miRNAs are small (~ 22 base pairs) noncoding RNA molecules that, when binding to target messenger RNAs (mRNAs), reduce their stability, leading to premature degradation or inhibition of their translation into proteins. Besides influencing the processes of myogenesis (Chen et al., 2006) and mitochondrial biogenesis (Russell et al., 2014), exercise has been shown to cause changes in the pattern of expression of several miRNAs (Radom-aizik et al., 2010; Eynon et al., 2011b; Kaliman et al., 2011), and some individuals respond better than others to exercise stimuli (Ehlert et al., 2013). The so-called lncRNA are bigger (> 200 bp) and are involved in transcriptional and post-transcriptional regulation. They control important cellular processes, such as the differentiation of precursor myoblasts (Ballarino et al., 2015). Additionally, some RNAs annotated as lncRNA might actually encode micropeptides. A very elegant study has recently identified a lncRNA responsible for encoding the micropeptide myoregulin, which inhibits the uptake of calcium into the sarcoplasmic reticulum. Genetic knock down of myoregulin improved physical performance in mice (Anderson et al., 2015). Furthermore, changes in the patterns of DNA or histone methylation can affect mRNA transcription by preventing binding of transcription factors to their targets. In general, exercise in sedentary individuals seems to generate a hypomethylation of the genome in skeletal muscle cells, increasing local gene expression (Ntanasis-Stathopoulos et al., 2013). Other mechanisms, such as chromatin condensation and remodeling, also regulate gene expression (Konopka & Sreekumaran Nair, 2013). Of note, given that epigenetic changes are used to regulate tissue-specific gene expression, epigenetic alterations found in blood cells in response to exercise might not reflect similar changes in muscle fibers or endothelial cells, although they may reflect important physiological changes or can indirectly serve as useful biomarkers in particular cases. It also should be noted that epigenetic changes may be long-lasting (Kaliman et al., 2011; Mangino, 2014) and may help explain the influence of physical activity on the longevity and muscle fiber composition of individuals. Moreover, some of these epigenetic changes may be transmitted to offspring (Ahmetov et al., 2012).

The next generation of genetic analyses

The greater availability of "next-generation sequencing" (NGS) techniques has enabled the emergence of whole genome studies aimed at understanding the different

phenotypes observed at the cellular, systemic, and behavioral levels (Ware et al., 2013). Having the ability to generate enormous amounts of raw data, NGS technologies have many applications, including massive identification of genetic polymorphisms and variable regions in the population(s) of interest, analysis of whole genomes and transcriptomes, and studies of epigenetic modifications on a grand scale (Cullum et al., 2011). Despite the seemingly high costs for a single analysis, these technologies actually represent a reduction in the total cost of sequencing when the amount of data that can be generated is considered (Xuan et al., 2013). Many more genetic markers can be typed simultaneously and the amount of information makes for more effective genetic association studies.

Exercise genomics has been incorporating these novel technologies, although more slowly than other fields of medical research (Bouchard, 2011; Mikami et al., 2014). Compared with other research areas, the number of published studies in the field of sports genetics still represents a very small fraction of the total of publications in the fields of sports and physical activity (Fig. 2(a)). A literature search in February 2015 in the Scopus database showed that the proportion of published studies containing terms commonly used in genetics studies (defined in our search as "genetic," "genome," "genomic," or "polymorphism") was lower in "sports or physical activity" studies than in other health sciences studies ("cancer," "diabetes," and "ischemic stroke") for most terms. Despite the small number of genetic studies on "sports or physical activity," the proportion of such studies has increased considerably over the last years, especially from 2011 onwards (Fig. 2(b)).

With increased use of these technologies, the systematic study of complete sequences of the DNA of high-performance athletes can help reveal which polymorphisms are related to a variety of sports disciplines. However, the costs of whole genome sequencing still are quite high. Alternatively, researchers can opt for only performing exome sequencing, i.e., the sequencing of the coding regions of the genome (van Dijk et al., 2014). Since the exome constitutes slightly less than about 1.5% of the human genome, it is far more feasible and economical to focus sequencing efforts on the exome. By associating these data with structural data from proteins, it is possible to understand how sequence changes could cause structural changes in proteins, affecting metabolic pathways related to high performance. However, exome sequencing will miss information in noncoding regions that could have substantial influence of performance.

Taking into account the importance of mitochondrial metabolism to athletic performance and the presence of a large number of mitochondria with multiple genome copies per cell, the assessment of the entire mitochondrial genome by NGS will permit a reliable elucidation of complete sequences, haplotypes, and haplogroups, and the identification of different types of mitochondrial genomes (heteroplasmy) in the same individual. In addition, differences in heteroplasmy levels among tissues may exist, highlighting the importance of choosing the appropriate tissue sample for each experiment (Tang et al., 2013; Wong, 2013). Although peripheral lymphocytes and buccal epithelial cells can be easily and quickly collected, their mitochondrial genomes may not reflect the same alterations related to heteroplasmy in muscle fibers. Different "mitochondrial populations" may have different metabolic phenotypes that can have an influence on the performance.

Looking for gene expression, unlike technologies such as quantitative polymerase chain reaction and microarrays, which allow the evaluation of selected panels of mRNA or microRNA, NGS can assess a wide

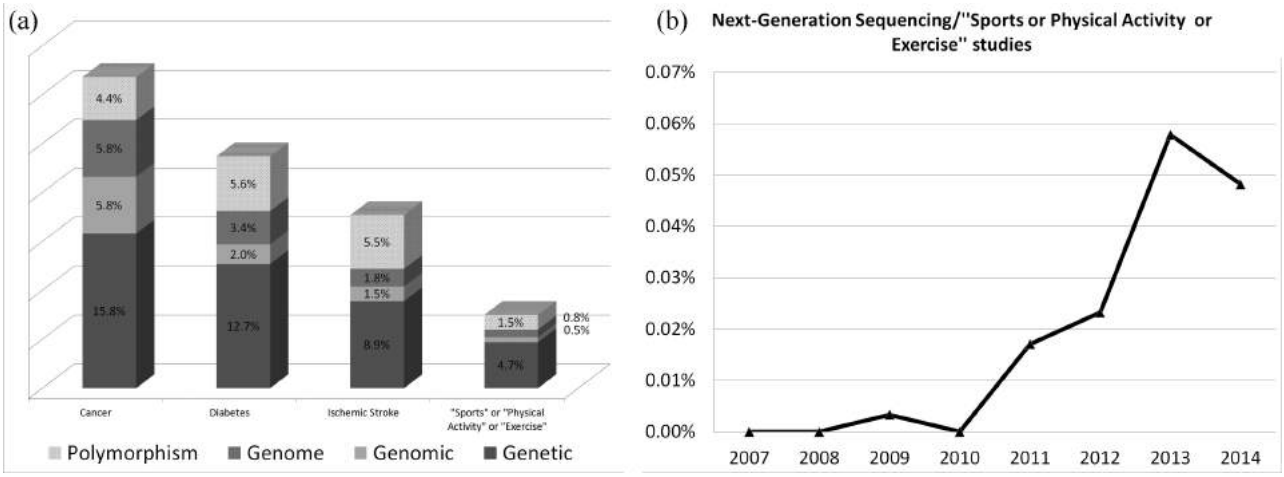

Fig. 2. Literature search showing the underrepresentation of genetic studies in the field of sports and physical activity. A literature search was performed in the Scopus database in February 2015. There were no language restrictions. (a) The bars indicate the percentage of published studies on each health science areas by cross-searching the terms "polymorphism," "genome," "genomic," and "genetic" with the terms "cancer," "diabetes," "ischemic stroke," and "sports or physical activity or exercise." (b) Percentage of published studies with the term "next-generation sequencing" among the studies on "sports or physical activity or exercise."

range of mRNA transcripts or microRNAs in a sample. Thus, a global overview can be obtained of gene expression and its variation after a biological stimulus, such as strength training. Regarding epigenetic factors, NGS can unveil DNA methylation patterns, post-translational editing, DNA–protein interactions, and transcription factor bindings, without restrictions to certain positions in the genome (Pareek et al., 2011).

NGS also can be coupled with modern separation techniques, allowing the evaluation of the genome, epigenome, or transcriptome of single cells (Raue et al., 2012; Murach et al., 2014). Thus, single-cell genetic analysis can contribute to the improvement in the understanding of several aspects of muscle cell function, regeneration, plasticity, and adaptation to physical activity, as well as to the comprehension of the unique characteristics of each type of muscle fiber.

In the near future, NGS tools could be used to generate important information about the contribution and interaction of a large number of genes and their variation in determining complex traits related to sports performance. However, the huge amount of data generated with the use of these new technologies requires a more rigorous analysis, greater caution with experimental design, and larger sample size to reduce false associations from being made.

Genomic studies for physical activity should strictly define and observe the performance influencing characteristics of each sport or activity to clearly determine the study variables. In complex traits, the variable (e.g., athletic parameters) must be properly selected so that sampling errors do not confound or overestimate the influence of a particular gene variant for sports performance. Moreover, genetic tests may have little or no prediction for athletic performance if inadequate parameters are selected.

By combining the use of the robust molecular tools with a sound experimental design that uses classical and well-established sports performance tests applied to a population with well-defined physiological variables, it will be possible to generate data of substantial scientific value. Good science can pave the way to understand what makes a high-performance athlete.

Perspectives

The evolution of molecular techniques may make it possible to uncover the contribution of the genetic component to physical performance. The association of genomic, transcriptomic, and epigenomic new sequencing technologies with proteomics/metabolomics data; the formation of international consortia to acquire thousands of samples; and the use of robust experimental designs are crucial steps to advance our knowledge about the molecular basis of sports performance.

Key words: Genetic, physical performance, genomic, heritability, polymorphisms, next-generation sequencing.

Acknowledgements

Professor Rosane Silva receives grants from Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ), and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES). Professor Rodrigo S Moura-Neto has a grant from FAPERJ and Professor Fernando A M S Pompeu has a grant from CAPES. Dr Pedro M Pimentel-Coelho is supported by a fellowship from Programa Ciência Sem Fronteiras-CAPES. The authors wish to thank Bruno Felipe Martins de Almeida for the artwork in Fig. 1.

References

Ahmetov II, Astratenkova IV, Rogozkin VA. Association of a PPARD polymorphism with human physical performance. Mol Biol 2007: 41: 776–780.

Ahmetov II, Druzhevskaya AM, Lyubaeva EV, Popov DV, Vinogradova OL, Williams AG. The dependence of preferred competitive racing distance on muscle fibre type composition and ACTN3 genotype in speed skaters. Exp Physiol 2011: 96: 1302–1310.

Ahmetov II, Gavrilov DN, Astratenkova IV, Druzhevskaya AM, Malinin AV, Romanova EE, Rogozkin VA. The association of ACE, ACTN3 and PPARA gene variants with strength phenotypes in middle school-age children. J Physiol Sci 2013: 63: 79–85.

Ahmetov II, Khakimullina AM, Popov DV, Missina SS, Vinogradova OL, Rogozkin VA. Polymorphism of the vascular endothelial growth factor gene (VEGF) and aerobic performance in athletes. Hum Physiol 2008: 34: 477–481.

Ahmetov II, Vinogradova OL, Williams AG. Gene polymorphisms and fiber-type composition of human skeletal muscle. Int J Sport Nutr Exerc Metab 2012: 22: 292–303.

Ahmetov II, Williams AG, Popov DV, Lyubaeva EV, Hakimullina AM, Fedotovskaya ON, Mozhayskaya IA, Vinogradova OL, Astratenkova IV, Montgomery HE, Rogozkin VA. The combined impact of metabolic gene polymorphisms on elite endurance athlete status and related phenotypes. Hum Genet 2009: 126: 751–761.

Alfred T, Ben-shlomo ÃY, Cooper R, Hardy R, Cooper C, Deary IJ, Gunnell D, Harris SE, Kumari M, Martin RM, Moran CN, Pitsiladis YP, Ring SM, Sayer AA, Smith GD, Starr JM, Kuh D, Day INM. ACTN3 genotype, athletic status, and life course physical capability: meta-analysis of the published literature and findings from nine studies. Hum Mutat 2011: 32: 1008–1018.

Amir O, Amir R, Yamin C, Attias E, Eynon N, Sagiv M, Sagiv M, Meckel Y. The ACE deletion allele is associated with Israeli elite endurance athletes. Exp Physiol 2007: 92: 881–886.

Anderson DM, Anderson KM, Chang CL, Makarewich CA, Nelson BR, McAnally JR, Kasaragod P, Shelton JM, Liou J, Bassel-Duby R, Olson EN.

A micropeptide encoded by a putative long noncoding RNA regulates muscle performance. Cell 2015: 160: 595–606.
Artioli GG, Dominguez R, Hirata C, Lancha H. Terapia gênica, doping genético e esporte: fundamentação e implicações para o futuro. Rev Bras Med 2007: 13: 349–354.
Ash GI, Scott RA, Deason M, Dawson TA, Wolde B, Bekele Z, Teka S, Pitsiladis YP. No association between ACE gene variation and endurance athlete status in Ethiopians. Med Sci Sports Exerc 2011: 43: 590–597.
Ballarino M, Cazzella V, D'Andrea D, Grassi L, Bisceglie L, Cipriano A, Santini T, Pinnaro C, Morlando M, Tramontano A, Bozzoni I. Novel long noncoding RNAs (lncRNAs) in myogenesis: a miR-31 overlapping lncRNA transcript controls myoblast differentiation. Mol Cell Biol 2015: 35: 728–736.
Ben-Zaken S, Meckel Y, Nemet D, Eliakim A. Genetic score of power-speed and endurance track and field athletes. Scand J Med Sci Sports 2015: 25: 166–174.
Berman Y, North KN. A gene for speed: the emerging role of alpha-actinin-3 in muscle metabolism. Physiology (Bethesda) 2010: 25: 250–259.
Bouchard C. Overcoming barriers to progress in exercise genomics. Exerc Sport Sci Rev 2011: 39: 212–217.
Bouchard C. Genomic predictors of trainability. Exp Physiol 2012: 97: 347–352.
Bouchard C, Daw EW, Rice T, Perusse L, Gagnon J, Province MA, Leon AS, Rao DC, Skinner JS, Wilmore JH. Familial resemblance for VO2max in the sedentary state: the HERITAGE family study. Med Sci Sports Exerc 1998: 30: 252–258.
Bouchard C, Malina RM, Pérusse L. Genetics of fitness and physical performance. Champaign, IL: Human Kinetics, 1997.
Bouchard C, Rankinen T, Timmons JA. Genomics and genetics in the biology of adaptation to exercise. Compr Physiol 2011a: 1: 1603–1648.
Bouchard C, Sarzynski MA, Rice TK, Kraus WE, Church TS, Sung YJ, Rao DC, Rankinen T. Genomic predictors of the maximal O_2 uptake response to standardized exercise training programs. J Appl Physiol (1985) 2011b: 110: 1160–1170.
Bray MS, Hagberg JM, Perusse L, Rankinen T, Roth SM, Wolfarth B, Bouchard C. The human gene map for performance and health-related fitness phenotypes: the 2006–2007 update. Med Sci Sports Exerc 2009: 41: 35–73.
Broos S, Malisoux L, Theisen D, Francaux M, Deldicque L, Thomis MA. Role of alpha-actinin-3 in contractile properties of human single muscle fibers: a case series study in paraplegics. PLoS ONE 2012: 7: e49281.
Buxens A, Ruiz JR, Arteta D, Artieda M, Santiago C, González-Freire M, Martínez A, Tejedor D, Lao JI, Gómez-Gallego F, Lucia A. Can we predict top-level sports performance in power vs endurance events? A genetic approach. Scand J Med Sci Sports 2010: 21: 570–579.
Calvo M, Rodas G, Vallejo M, Estruch A, Arcas A, Javierre C, Viscor G, Ventura J. Heritability of explosive power and anaerobic capacity in humans. Eur J Appl Physiol 2002: 86: 218–225.
Cerit M, Colakoglu M, Erdogan M, Berdeli A, Cam FS. Relationship between ace genotype and short duration aerobic performance development. Eur J Appl Physiol 2006: 98: 461–465.
Charbonneau DE, Hanson ED, Ludlow AT, Delmonico MJ, Hurley BF, Roth SM. ACE genotype and the muscle hypertrophic and strength responses to strength training. Med Sci Sports Exerc 2008: 40: 677–683.
Chen JF, Mandel EM, Thomson JM, Wu Q, Callis TE, Hammond SM, Conlon FL, Wang DZ. The role of microRNA-1 and microRNA-133 in skeletal muscle proliferation and differentiation. Nat Genet 2006: 38: 228–233.
Cięszczyk P, Eider J, Ostanek M, Arczewska A, Leońska-Duniec A, Sawczyn S, Ficek K, Krupecki K. Association of the *ACTN3* R577X Polymorphism in Polish power-orientated athletes. J Hum Kinet 2011: 28: 55–61.
Costa AM, Breitenfeld L, Silva AJ, Pereira A, Izquierdo M, Marques MC. Genetic inheritance effects on endurance and muscle strength: an update. Sports Med 2012: 42: 449–458.
Cullum R, Alder O, Hoodless PA. The next generation: using new sequencing technologies to analyse gene regulation. Respirology 2011: 16: 210–222.
de Groote P, Lamblin N, Helbecque N, Mouquet F, Hermant X, Amouyel P, Dallongeville J, Bauters C. The impact of the AMPD1 gene polymorphism on exercise capacity, other prognostic parameters, and survival in patients with stable congestive heart failure: a study in 686 consecutive patients. Am Heart J 2006: 152: 736–741.
Deason M, Scott R, Irwin L, Macaulay V, Fuku N, Tanaka M, Irving R, Charlton V, Morrison E, Austin K, Pitsiladis YP. Importance of mitochondrial haplotypes and maternal lineage in sprint performance among individuals of West African ancestry. Scand J Med Sci Sports 2012: 22: 217–223.
Denham J, Marques FZ, O'Brien BJ, Charchar FJ. Exercise: putting action into our epigenome. Sports Med 2014: 44: 189–209.
Dhamrait SS, Williams AG, Day SH, Skipworth J, Payne JR, World M, Humphries SE, Montgomery HE. Variation in the uncoupling protein 2 and 3 genes and human performance. J Appl Physiol (1985) 2012: 112: 1122–1127.
Dias RG. Genética, Performance Física Humana e Doping Genético: o Senso Comum Versus a Realidade Científica. Rev Bras Med 2011: 17: 62–70.
Ehlert T, Simon P, Moser DA. Epigenetics in sports. Sports Med 2013: 43: 93–110.
Erskine RM, Jones DA, Williams AG, Stewart CE, Degens H. Resistance training increases *in vivo* quadriceps femoris muscle specific tension in young men. Acta Physiol 2010: 199: 83–89.
Erskine RM, Williams AG, Jones DA, Stewart CE, Degens H. The individual and combined influence of ACE and ACTN3 genotypes on muscle phenotypes before and after strength training. Scand J Med Sci Sports 2014: 24: 642–648.
Eynon N, Alves AJ, Meckel Y, Yamin C, Ayalon M, Sagiv M, Sagiv M. Is the interaction between HIF1A P582S and ACTN3 R577X determinant for power/sprint performance? Metabolism 2010a: 59: 861–865.
Eynon N, Birk R, Meckel Y, Lucia A, Nemet D, Eliakim A. Physiological variables and mitochondrial-related genotypes of an athlete who excels in both short and long-distance running. Mitochondrion 2011a: 11: 774–777.
Eynon N, Hanson ED, Lucia A, Houweling PJ, Garton F, North KN, Bishop DJ. Genes for elite power and sprint performance: ACTN3 leads the way. Sports Med 2013: 43: 803–817.
Eynon N, Meckel Y, Sagiv M, Yamin C, Amir R, Goldhammer E, Duarte JA, Oliveira J. Do PPARGC1A and PPARalpha polymorphisms influence sprint or endurance phenotypes? Scand J Med Sci Sports 2010b: 20: e145–e150.
Eynon N, Ruiz J, Oliveira J, Duarte JA, Birk R, Lucia A. Genes and elite athletes: a roadmap for future research. J Physiol 2011b: 13: 3063–3070.
Eynon N, Ruiz JR, Femia P, Pushkarev VP, Cieszczyk P, Maciejewska-Karlowska A, Sawczuk M, Dyatlov DA, Lekontsev EV, Kulikov LM, Birk R, Bishop DJ, Lucia A. The ACTN3 R577X polymorphism

across three groups of elite male European athletes. PLoS ONE 2012: 7: e43132–e43132.
Eynon N, Ruiz JR, Meckel Y, Morán M, Lucia A. Mitochondrial biogenesis related endurance genotype score and sports performance in athletes. Mitochondrion 2011c: 11: 64–69.
Eynon N, Ruiz JR, Oliveira J, Duarte JA, Birk R, Lucia A. Genes and elite athletes: a roadmap for future research. J Physiol 2011d: 589: 3063–3070.
Fagard R, Bielen E, Amery A. Heritability of aerobic power and anaerobic energy generation during exercise. J Appl Physiol (1985) 1991: 70: 357–362.
Flueck M, Eilers W. Training modalities: impact on endurance capacity. Endocrinol Metab Clin North Am 2010: 39: 183–200, xi.
Ginevičienė V, Pranckevičienė E, Milašius K, Kučinskas V. Gene variants related to the power performance of the Lithuanian athletes. Cent Eur J Biol 2011: 6: 48–57.
Gomez-Pinilla F, Zhuang Y, Feng J, Ying Z, Fan G. Exercise impacts brain-derived neurotrophic factor plasticity by engaging mechanisms of epigenetic regulation. Eur J Neurosci 2011: 33: 383–390.
Gordon SE, Davis BS, Carlson CJ, Booth FW. ANG II is required for optimal overload-induced skeletal muscle hypertrophy. Am J Physiol Endocrinol Metab 2001: 280: E150–E159.
Grealy R, Smith CLE, Chen T, Hiller D, Haseler LJ, Griffiths LR. Journal of Science and Medicine in Sport The genetics of endurance: frequency of the ACTN3 R577X variant in Ironman World Championship athletes. J Sci Med Sport 2013: 16: 365–371.
Hagberg JM, Rankinen T, Loos RJF, Pérusse L, Roth SM, Wolfarth B, Bouchard C. Advances in exercise, fitness, and performance genomics in 2010. Med Sci Sports Exerc 2011: 43: 743–752.
He ZH, Hu Y, Li YC, Yvert T, Santiago C, Gomez-Gallego F, Ruiz JR, Lucia A. Are calcineurin genes associated with athletic status? A function, replication study. Med Sci Sports Exerc 2011: 43: 1433–1440.
Henderson J, Withford-Cave JM, Duffy DL, Cole SJ, Sawyer NA, Gulbin JP, Hahn A, Trent RJ, Yu B. The EPAS1 gene influences the aerobic-anaerobic contribution in elite endurance athletes. Hum Genet 2005: 118: 416–423.
Holdys J, Kryściak J, Stanisławski D, Gronek P. ACE I/D gene polymorphism in athletes of various sports disciplines. Human Movement 2011: 12: 223–231.
Hong EP, Park JW. Sample size and statistical power calculation in genetic association studies. Genomics Inform 2012: 10: 117–122.
Hong SS, Jin HJ. Assessment of association of ACTN3 genetic polymorphism with Korean elite athletic performance. Genes Genomics 2013: 35: 617–621.
Hubal MJ, Gordish-Dressman H, Thompson PD, Price TB, Hoffman EP, Angelopoulos TJ, Gordon PM, Moyna NM, Pescatello LS, Visich PS, Zoeller RF, Seip RL, Clarkson PM. Variability in muscle size and strength gain after unilateral resistance training. Med Sci Sports Exerc 2005: 37: 964–972.
Jannot AS, Ehret G, Perneger T. $P < 5 \times 10(-8)$ has emerged as a standard of statistical significance for genome-wide association studies. J Clin Epidemiol 2015: 68: 460–465.
Jiang LQ, Garcia-Roves PM, de Castro Barbosa T, Zierath JR. Constitutively active calcineurin in skeletal muscle increases endurance performance and mitochondrial respiratory capacity. Am J Physiol Endocrinol Metab 2010: 298: E8–E16.
Kaliman P, Párrizas M, Lalanza JF, Camins A, Escorihuela RM, Pallàs M. Neurophysiological and epigenetic effects of physical exercise on the aging process. Ageing Res Rev 2011: 10: 475–486.
Keller P, Vollaard NB, Gustafsson T, Gallagher IJ, Sundberg CJ, Rankinen T, Britton SL, Bouchard C, Koch LG, Timmons JA. A transcriptional map of the impact of endurance exercise training on skeletal muscle phenotype. J Appl Physiol (1985) 2011: 110: 46–59.
Kim J, Oh S, Min H, Kim Y, Park T. Practical issues in genome-wide association studies for physical activity. Ann N Y Acad Sci 2011: 1229: 38–44.
Komi PV, Klissouras V, Karvinen E. Genetic variation in neuromuscular performance. Int Z Angew Physiol 1973: 31: 289–304.
Konopka AR, Sreekumaran Nair K. Mitochondrial and skeletal muscle health with advancing age. Mol Cell Endocrinol 2013: 379: 19–29.
Larsson NG, Oldfors A. Mitochondrial myopathies. Acta Physiol Scand 2001: 171: 385–393.
Lek M, Quinlan KGR, North KN. The evolution of skeletal muscle performance: gene duplication and divergence of human sarcomeric alpha-actinins. Bioessays 2010: 32: 17–25.
Lewin B. Genes IX. Porto Alegre: Artmed, 2009.
Lucia A, Martin MA, Esteve-Lanao J, San Juan AF, Rubio JC, Oliván J, Arenas J. C34T mutation of the AMPD1 gene in an elite white runner. Br J Sports Med 2006: 40: e7–e7.
Ma F, Yang Y, Li X, Zhou F, Gao C, Li M, Gao L. The association of sport performance with ACE and ACTN3 genetic polymorphisms: a systematic review and meta-analysis. PLoS One 2013: 8: e54685.
Macarthur DG, Seto JT, Chan S, Quinlan KGR, Joanna M, Turner N, Nicholson MD, Kee AJ, Hardeman EC, Peter W, Cooney GJ, Head SI, Yang N, North KN. An Actn3 knockout mouse provides mechanistic insights into the association between a -actinin-3 deficiency and human athletic performance. Hum Mol Genet 2008: 17: 1076–1086.
MacArthur DG, North KN. ACTN3: a genetic influence on muscle function and athletic performance. Exerc Sport Sci Rev 2007: 35: 30–34.
Maciejewska A, Sawczuk M, Cięszczyk P. Variation in the PPARα gene in Polish rowers. J Sci Med Sport 2011: 14: 58–64.
Maciejewska A, Sawczuk M, Cieszczyk P, Mozhayskaya IA, Ahmetov II. The PPARGC1A gene Gly482Ser in Polish and Russian athletes. J Sports Sci 2012: 30: 101–113.
Maciejewska-Karlowska A, Hanson ED, Sawczuk M, Cieszczyk P, Eynon N. Genomic haplotype within the peroxisome proliferator-activated receptor delta (PPARD) gene is associated with elite athletic status. Scand J Med Sci Sports 2014: 24: e148–e155.
Mangino M. Genomics of ageing in twins. Proc Nutr Soc 2014: 73: 526–531.
Manolio TA. Genomewide association studies and assessment of the risk of disease. N Engl J Med 2010: 363: 166–176.
Martínez JL, Khorsandi S, Sojo R, Martínez C, Martín JA, López-Taylor JR, Fahey TD, Rivera MA. Lack of an association between CKMM Genotype and endurance performance level in Hispanic marathon runners. Medicina Sportiva 2009: 13: 219–223.
Maruszak A, Adamczyk JG, Siewierski M, Sozański H, Gajewski A, Zekanowski C. Mitochondrial DNA variation is associated with elite athletic status in the Polish population. Scand J Med Sci Sports 2014: 24: 311–318.
McCauley T, Mastana SS, Hossack J, Macdonald M, Folland JP. Human angiotensin-converting enzyme I/D and alpha-actinin 3 R577X genotypes and muscle functional and contractile properties. Exp Physiol 2008: 94: 81–89.
McGee SL, Hargreaves M. Histone modifications and exercise adaptations

signals mediating skeletal muscle remodeling by activity histone modifications and exercise adaptations. J Appl Physiol 2011: 110: 258–263.
Mikami E, Fuku N, Murakami H, Tsuchie H, Takahashi H, Ohiwa N, Tanaka H, Pitsiladis YP, Higuchi M, Miyachi M, Kawahara T, Tanaka M. ACTN3 R577X genotype is associated with sprinting in elite Japanese athletes. Int J Sports Med 2014: 35: 172–177.
Mikami E, Fuku N, Takahashi H, Ohiwa N, Scott RA, Pitsiladis YP, Higuchi M, Kawahara T, Tanaka M. Mitochondrial haplogroups associated with elite Japanese athlete status. Br J Sports Med 2011: 45: 1179–1183.
Moor MHMD, Spector TD, Cherkas LF, Falchi M, Hottenga JJ, Boomsma DI, Geus EJCD. Genome-wide linkage scan for athlete status in 700 British female DZ twin pairs. Twin Res Hum Genet 2007: 10: 812–820.
Murach K, Raue U, Wilkerson B, Minchev K, Jemiolo B, Bagley J, Luden N, Trappe S. Single muscle fiber gene expression with run taper. PLoS ONE 2014: 9: e108547.
Mustelin L, Latvala A, Pietiläinen KH, Piirilä P, Sovijärvi AR, Kujala UM, Rissanen A, Kaprio J. Associations between sports participation, cardiorespiratory fitness, and adiposity in young adult twins. J Appl Physiol (1985) 2011: 110: 681–686.
Myburgh KH. What makes an endurance athlete world-class? Not simply a physiological conundrum. Comp Biochem Physiol A Mol Integr Physiol 2003: 136: 171–190.
Niemi AK, Majamaa K. Mitochondrial DNA and ACTN3 genotypes in Finnish elite endurance and sprint athletes. Eur J Hum Genet 2005: 13: 965–969.
Nogales-Gadea G, Pinós T, Ruiz JR, Marzo PF, Fiuza-Luces C, López-Gallardo E, Ruiz-Pesini E, Martín MA, Arenas J, Morán M, Andreu AL, Lucia A. Are mitochondrial haplogroups associated with elite athletic status? A study on a Spanish cohort. Mitochondrion 2011: 11: 905–908.
Norman B, Esbjörnsson M, Rundqvist H, Osterlund T, von Walden F, Tesch PA. Strength, power, fiber types, and mRNA expression in trained men and women with different ACTN3 R577X genotypes. J Appl Physiol (1985) 2009: 106: 959–965.
North K. Why is alpha-actinin-3 deficiency so common in the general population? The evolution of athletic performance. Twin Res Hum Genet 2008: 11: 384–394.
North K, Yang N, Wattanasirichaigoon D, Mills M, Easteal S, Beggs AH. A common nonsense mutation results in α-actinin-3 deficiency. Nat Genet 1999: 21: 353–354.
Ntanasis-Stathopoulos J, Tzanninis JG, Philippou A, Koutsilieris M. Epigenetic regulation on gene expression induced by physical exercise. J Musculoskelet Neuronal Interact 2013: 13: 133–146.
Nunnari J, Suomalainen A. Mitochondria: in sickness and in health. Cell 2012: 148: 1145–1159.
Onywera VO, Scott RA, Boit MK, Pitsiladis YP. Demographic characteristics of elite Kenyan endurance runners. J Sports Sci 2006: 24: 415–422.
Pareek CS, Smoczynski R, Tretyn A. Sequencing technologies and genome sequencing. J Appl Genet 2011: 52: 413–435.
Pérusse L, Rankinen T, Hagberg JM, Loos RJF, Roth SM, Sarzynski MA, Wolfarth B, Bouchard C. Advances in exercise, fitness, and performance genomics in 2012. Med Sci Sports Exerc 2013: 45: 824–831.
Pescatello LS, Devaney JM, Hubal MJ, Thompson PD, Hoffman EP. Highlights from the functional single nucleotide polymorphisms associated with human muscle size and strength or FAMuSS study. Biomed Res Int 2013: 2013: 1–11.
Pitsiladis Y, Wang G. Necessary advances in exercise genomics and likely pitfalls. J Appl Physiol (1985) 2011: 110: 1150–1151.
Pitsiladis Y, Wang G, Wolfarth B, Scott R, Fuku N, Mikami E, He Z, Fiuza-luces C, Eynon N, Lucia A. Genomics of elite sporting performance: what little we know and necessary advances. Br J Sports Med 2013: 47: 550–555.
Radom-aizik S, Zaldivar F, Oliver S, Galassetti P, Cooper DM, Oliver S, Galassetti P, Dm C. Evidence for microRNA involvement in exercise-associated neutrophil gene expression changes. J Appl Physiol (1985) 2010: 109: 252–261.
Raleigh SM. Epigenetic regulation of the ACE gene might be more relevant to endurance physiology than the I/D polymorphism. J Appl Physiol (1985) 2012: 112: 1082–1083.
Rankinen T, Bray MS, Hagberg JM, Pérusse L, Roth SM, Wolfarth B, Bouchard C. The human gene map for performance and health-related fitness phenotypes: the 2005 update. Med Sci Sports Exerc 2006: 38: 1863–1888.
Raue U, Trappe TA, Estrem ST, Qian HR, Helvering LM, Smith RC, Trappe S. Transcriptome signature of resistance exercise adaptations: mixed muscle and fiber type specific profiles in young and old adults. J Appl Physiol (1985) 2012: 112: 1625–1636.
Rezk BM, Yoshida T, Semprun-Prieto L, Higashi Y, Sukhanov S, Delafontaine P. Angiotensin II infusion induces marked diaphragmatic skeletal muscle atrophy. PLoS ONE 2012: 7: e30276.
Rigat B, Hubert C, Alhenc-gelas F, Cambien F, Corvol P, Soubrier F. An insertion/deletion polymorphism in the angiotensin I-converting enzyme gene accounting for half the variance of serum enzyme levels. J Clin Invest 1990: 86: 1343–1346.
Rubio JC, Martín MA, Rabadán M, Gómez-Gallego F, San Juan AF, Alonso JM, Chicharro JL, Pérez M, Arenas J, Lucia A. Frequency of the C34T mutation of the AMPD1 gene in world-class endurance athletes: does this mutation impair performance? J Appl Physiol (1985) 2005: 98: 2108–2112.
Ruiz JR, Arteta D, Buxens A, Artieda M, Gomez-Gallego F, Santiago C, Yvert T, Moran M, Lucia A. Can we identify a power-oriented polygenic profile? J Appl Physiol (1985) 2010: 108: 561–566.
Ruiz JR, Gómez-Gallego F, Santiago C, González-Freire M, Verde Z, Foster C, Lucia A. Is there an optimum endurance polygenic profile? J Physiol 2009: 587: 1527–1534.
Russell AP, Foletta VC, Snow RJ, Wadley GD. Skeletal muscle mitochondria: a major player in exercise, health and disease. Biochim Biophys Acta 2014: 1840: 1276–1284.
Sanchis-Gomar F, Garcia-Gimenez J, Perez-Quilis C, Gomez-Cabrera M, Pallardo F, Lippi G. Physical exercise as an epigenetic modulator: eustress, the "positive stress" as an effector of gene expression. J Strength Cond Res 2012: 26: 3469–3472.
Santiago C, Ruiz JR, Muniesa CA, González-Freire M, Gómez-Gallego F, Lucia A. Does the polygenic profile determine the potential for becoming a world-class athlete? Insights from the sport of rowing. Scand J Med Sci Sports 2010: 20: e188–e194.
Santiago C, Ruiz JR, Rodríguez-Romo G, Fiuza-Luces C, Yvert T, Gonzalez-Freire M, Gómez-Gallego F, Morán M, Lucia A. The K153R polymorphism in the myostatin gene and muscle power phenotypes in young, non-athletic men. PLoS ONE 2011: 6: e16323–e16323.
Saunders CJ, Xenophontos SL, Cariolou MA, Anastassiades LC, Noakes TD, Collins M. The bradykinin beta 2 receptor (BDKRB2) and endothelial nitric oxide synthase 3 (NOS3) genes

and endurance performance during Ironman Triathlons. Hum Mol Genet 2006: 15: 979–987.
Schiaffino S, Reggiani C. Fiber types in mammalian skeletal muscles. Physiol Rev 2011: 91: 1447–1531.
Scott RA, Fuku N, Onywera VO, Boit M, Wilson RH, Tanaka M, H Goodwin W, Pitsiladis YP. Mitochondrial haplogroups associated with elite Kenyan athlete status. Med Sci Sports Exerc 2008: 41: 123–128.
Scott RA, Irving R, Irwin L, Morrison E, Charlton V, Austin K, Tladi D, Deason M, Headley SA, Kolkhorst FW, Yang N, North K, Pitsiladis YP. ACTN3 and ACE genotypes in elite Jamaican and US sprinters. Med Sci Sports Exerc 2010: 42: 107–112.
Scott RA, Moran C, Wilson RH, Onywera V, Boit MK, Goodwin WH, Gohlke P, Payne J, Montgomery H, Pitsiladis YP. No association between Angiotensin Converting Enzyme (ACE) gene variation and endurance athlete status in Kenyans. Comp Biochem Physiol A Mol Integr Physiol 2005a: 141: 169–175.
Scott RA, Pitsiladis YP. Genotypes and distance running: clues from Africa. Sports Med 2007: 37: 424–427.
Scott RA, Wilson RH, Goodwin WH, Moran CN, Georgiades E, Wolde B, Pitsiladis YP. Mitochondrial DNA lineages of elite Ethiopian athletes. Comp Biochem Physiol B Biochem Mol Biol 2005b: 140: 497–503.
Semprun-Prieto LC, Sukhanov S, Yoshida T, Rezk BM, Gonzalez-Villalobos RA, Vaughn C, Michael Tabony A, Delafontaine P. Angiotensin II induced catabolic effect and muscle atrophy are redox dependent. Biochem Biophys Res Commun 2011: 409: 217–221.
Serrano NC, Díaz LA, Casas JP, Hingorani AD, Moreno de Lucca D, Páez MC. Frequency of eNOS polymorphisms in the Colombian general population. BMC Genet 2010: 11: 54–54.
Seto JT, Quinlan KG, Lek M, Zheng XF, Garton F, MacArthur DG, Hogarth MW, Houweling PJ, Gregorevic P, Turner N, Cooney GJ, Yang N, North KN. ACTN3 genotype influences muscle performance through the regulation of calcineurin signaling. J Clin Invest 2013: 123: 4255–4263.
Sharp NCC. The human genome and sport, including epigenetics, gene doping, and athleticogenomics. Endocrinol Metab Clin North Am 2010: 39: 201–215.
Stubbe JH, Boomsma DI, Vink JM, Cornes BK, Martin NG, Skytthe A, Kyvik KO, Rose RJ, Kujala UM, Kaprio J, Harris JR, Pedersen NL, Hunkin J, Spector TD, de Geus EJC. Genetic influences on exercise participation in 37 051 twin pairs from seven countries. PLoS ONE 2006: 1: e22–e22.
Sundet J, Magnus P, Tambs K. The heritability of maximal aerobic power: a study of Norwegian twins. Scand J Med Sci Sports 1994: 4: 181–185.
Tabony AM, Yoshida T, Galvez S, Higashi Y, Sukhanov S, Chandrasekar B, Mitch WE, Delafontaine P. Angiotensin II upregulates protein phosphatase 2Calpha and inhibits AMP-activated protein kinase signaling and energy balance leading to skeletal muscle wasting. Hypertension 2011: 58: 643–649.
Tang S, Wang J, Zhang VW, Li FY, Landsverk M, Cui H, Truong CK, Wang G, Chen LC, Graham B, Scaglia F, Schmitt ES, Craigen WJ, Wong LJ. Transition to next generation analysis of the whole mitochondrial genome: a summary of molecular defects. Hum Mutat 2013: 34: 882–893.
Tesch PA, Karlsson J. Muscle fiber types and size in trained and untrained muscles of elite athletes. J Appl Physiol (1985) 1985: 59: 1716–1720.
Thorn CF, Kleina TE, Altmana RB. PharmGKB summary: very important pharmacogene information for angiotensin-converting enzyme. Pharmacogenet Genomics 2010: 20: 143–146.
Tsianos GI, Evangelou E, Boot A, Zillikens MC, van Meurs JB, Uitterlinden AG, Ioannidis JP. Associations of polymorphisms of eight muscle- or metabolism-related genes with performance in Mount Olympus marathon runners. J Appl Physiol (1985) 2010: 108: 567–574.
van Dijk EL, Auger H, Jaszczyszyn Y, Thermes C. Ten years of next-generation sequencing technology. Trends Genet 2014: 30: 418–426.
Vincent B, De Bock K, Ramaekers M, Van den Eede E, Van Leemputte M, Hespel P, Thomis MA. ACTN3 (R577X) genotype is associated with fiber type distribution. Physiol Genomics 2007: 32: 58–63.
Ware JS, Petretto E, Cook SA. Integrative genomics in cardiovascular medicine. Cardiovasc Res 2013: 97: 623–630.
Wilber RL, Pitsiladis YP. Kenyan and Ethiopian distance runners: what makes them so good? Int J Sports Physiol Perform 2012: 7: 92–102.
Williams AG, Dhamrait SS, Wootton PTE, Day SH, Hawe E, Payne JR, Myerson SG, World M, Budgett R, Humphries SE, Montgomery HE. Bradykinin receptor gene variant and human physical performance. J Appl Physiol (1985) 2004: 96: 938–942.
Wong LJ. Challenges of bringing next generation sequencing technologies to clinical molecular diagnostic laboratories. Neurother 2013: 10: 262–272.
Xuan J, Yu Y, Qing T, Guo L, Shi L. Next-generation sequencing in the clinic: promises and challenges. Cancer Lett 2013: 340: 284–295.
Yang N, MacArthur DG, Wolde B, Onywera VO, Boit MK, Lau SYMA, Wilson RH, Scott RA, Pitsiladis YP, North K. The ACTN3 R577X polymorphism in East and West African athletes. Med Sci Sports Exerc 2007: 39: 1985–1988.
Yazdi SMZ, Robin NH. We need to know our limitations: genetic testing for complex traits. Curr Opin Pediatr 2013: 25: 643–644.
Zhang J, Chiodini R, Badr A, Zhang G. The impact of next-generation sequencing on genomics. J Genet Genomics 2011: 38: 95–109.
Zhou DQ, Hu Y, Liu G, Gong L, Xi Y, Wen L. Muscle-specific creatine kinase gene polymorphism and running economy responses to an 18-week 5000-m training programme. Br J Sports Med 2006: 40: 988–991.
Zilberman-schapira G, Chen J, Gerstein M, Samples C. On sports and genes. Recent Pat DNA Gene Seq 2012: 6: 1–9.
Zoossmann-Diskin A. The association of the ACE gene and elite athletic performance in Israel may be an artifact. Exp Physiol 2008: 93: 1220. author reply 1221.