



Genetic polymorphisms determining of the physical performance in elite athletes

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ABSTRACT

This article is focused on the review of studies looking for “candidate genes” and their relationship with physical performance phenotypes in elite athletes. Our goal is to bring to readers what makes some individuals excel in some sports modalities, based on variants in genetic loci and markers. In addition, we assume the necessity to describe by what mechanisms a gene can contribute in physical performance, detailing in each part the cellular, physiological and molecular pathways involved. For this reason, we limited our discussion to a small number of genetic variants: polymorphisms R577X α -actinin 3 gene (ACTN3), C34T AMP deaminase gene (AMPD1), I/D angiotensin converting enzyme gene (ACE), –9/+9 β_2 bradykinin receptor gene (BDKRB2), and 985+185/1170 creatine kinase M gene (CK-M). We hope that this article bring some new information and refine the knowledge to the fact that the process of talent identification and an individual athletic potential maximization resulting in sport success are strongly associated with genetic variants.

INTRODUCTION

Human physical performance phenomenon in specific sports modalities has always been target of interest for sports medicine specialists as well as exercise physiologists. These professionals confirm the outline performance levels of their athletes from morphological and functional analyses, making use of histochemical techniques, biochemical dosing as well as analysis of cardiopulmonary parameters for this matter. It was believed that the athletes' high performance levels were derived from specific training and nutritional follow-up, factors which are essential for the development of elite athletes characteristics. However, as time passed by, such environmental factors alone were insufficient in order to characterize a status phenotype in human physical performance. From this premise, an interest for a third determinant factor of this complex phenotype for physical fitness aroused, that is, the genetic predisposition which, if it is not the most important, has great implications in the characterization of an individual as a remarkable athlete.

The identification of the structure of the deoxyribonucleic acid (DNA), by James Watson and Francis Crick in 1953, and the rapid advance of molecular biology techniques made the identification

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of variant sequences in the DNA of specific genes possible, relating such gene heterogeneity to different phenotypes⁽¹⁾. The genetic differences based on polymorphisms, with potential to affect aptitude as well as human physical performance, started to be investigated in the 1990's⁽²⁾. An alteration in the sequence of the DNA bases of a gene which decodes a protein may influence both its expression and its activity. Therefore, a modification in the sequence of nucleotides in the 10 q24-q26 chromosome, that is, the gene which decodes the adrenoceptor α_{2A} , which when stimulated by catecholamines in the adipose tissue inhibits lipolysis, could alter the plasma levels of free fatty acids during exercise. Thus, a polymorphism which increases the affinity of this receptor by its binding, would reduce the substrate offer, suggesting an unsuitable supply to the energetic demand of endurance athletes in extended events.

The variability of the mechanical and biological responses of the different systems specific to elite athletes of each specific modality, enables the study of what is called in the genetics field as tracing of the 'candidate genes'⁽³⁾. Up to the present moment, it is known that there are 170 gene variant sequences and genetic markers which are related to the physical performance phenotypes as well as good physical fitness concerned with health in the human genetic map⁽⁴⁾. These discoveries seem to be revolutionary in the identification of talents, with the individual gene characterization as significant part in the selection of young talents⁽⁵⁾. Nevertheless, it is important highlighting that multiple biological and environmental factors are determinant of performance and that the analysis of a single gene in isolation does not necessarily determine the phenotype of an athlete.

Although a great number of genes and genetic markers have been already reported, showing the association with physical performance phenotypes and health-related good fitness, our aim is to bring to the reader knowledge on the importance of some genetic variants in the phenotypes concerned with human physical performance in elite athletes. Attention will be given to some genes with potential to influence athletes' performance in modalities which demand endurance or strength/muscular power, discussing in detail for each one of them, the biological mechanisms through which polymorphism contributes to the characterization of an individual as an elite athlete.

R577X POLYMORPHISM OF THE α -ACTIN 3 GENE (ACTN3)

A phenotype well-characterized in athletes of different modalities is the kind of fiber of the skeletal muscle. In adults, this phenotype is determined by the expression of three distinct genes which, when transcribed and translated, synthesize isoforms of high chain (MHC), partly determining thus the percentage distribution of the different types of fibers of the muscle. Approximately 45% of the variations of the fiber type in the muscle are explained by genetic factors⁽⁶⁾. This distribution constitutes one of the determinant performance factors in sports modalities.

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Regardless the heterogeneity and distribution of the different types of fiber in the skeletal muscles, the muscular contraction is dependent on the interaction of the myofibrillar proteins myosin and actin⁽⁷⁾. The structural organization as well as maintenance of the contractile muscular apparatus is also dependent on protein complexes which bind the sarcomeres among each other and sustain them in the muscular fiber. Within this context, the α -actin constitutes the predominant protein. It is a component of the sarcomeric Z line⁽⁵⁾, which belongs to the actin-binding proteins family, which is important in the anchoring of the actin myofilaments as well as maintenance of the myofibrillar organization⁽⁸⁾. Four genes for have been described in humans for the α -actin (ACTN1, 2, 3 and 4), being the isoforms 2 and 3 constituents of the muscular cytoskeleton⁽⁹⁾. It is also known that the ACTN3 isoform is specific from the fast contraction fibers (type II) responsible for the generation of contractile strength in high velocity⁽¹⁰⁻¹²⁾.

The switch of a nucleotide C \rightarrow T in the 1747 position of the exon 16 was identified in the ACTN3 gene, that is, a mutation resulting in the conversion of the arginine amino acid into a premature stop codon in the 577 residue (R577X)⁽¹³⁻¹⁴⁾. Individuals homozygote for the 577X allele do not express α -actin3⁽¹⁵⁾. Curiously, α -actin3 deficiency does not derive in a pathological phenotype as muscular dystrophy or myopathies⁽¹⁴⁾, suggesting that the ACTN2 isoform (81% of homology in the amino acids sequence) could compensate for the absence of the α -actin3⁽¹⁵⁾. Yang *et al.*⁽¹⁰⁾, demonstrated association between the different ACTN3 genotypes as well as performance in elite athletes. If the α -actin3 plays an important role in type II muscle fibers, it would be reasonable to predict differences in the skeletal muscular function among individuals with different genotypes (R577X) for ACTN3. For some authors⁽⁶⁾, individuals who express the ACTN3 gene (RR or RX genotypes) may present advantage in modalities which require explosion and muscular strength when compared with individuals with XX genotype. In order to test such hypothesis, Yang *et al.*⁽¹⁰⁾ compared the genotypes and the frequency of the alleles from 107 elite athletes in velocity/strength (72 males and 35 females), 194 elite athletes from endurance events (122 males and 72 females) and 436 non-athletes healthy individuals, all genotyped for the ACTN3 gene. These authors verified a significant difference in the frequency of alleles between velocity/strength athletes and the control individuals, both for males ($p < 0.001$) and females ($p < 0.01$). These athletes, when analyzed in the totality (72 males + 35 females = 107) presented lower frequency of the XX genotype when compared with the control individuals (6% vs. 18%, respectively). None of the 35 velocity/strength athletes (female sex) presented XX genotype. When analyzed in the totality, velocity/strength athletes (107 individuals) presented higher frequency of the RR genotype and lower frequency of the RX genotype (50% and 45%, respectively), comparing with the control group (39% and 52%, respectively). An interesting point in the study was the comparison between velocity/strength athletes and endurance athletes who showed frequency of alleles in opposite directions, being the values significantly different for both sexes. The frequency of the XX genotype in the male sex was 20% for endurance athletes and 8% for velocity/strength athletes; in the female sex, 29% for endurance athletes and 0% for velocity/strength athletes. The frequency of the RR genotype in the male sex was 28% for endurance athletes and 53% for velocity/strength athletes; in the female sex 36% for endurance athletes and 43% for velocity/strength athletes.

The apparent benefit of the presence of the 577R allele in velocity/strength athletes is consistent with the localization of the α -actin3 in the skeletal muscles fibers of rapid contraction. On the other hand, some authors⁽⁵⁾ suggest that the absence of the ACTN3 gene expression (XX genotype) would be related to better performance in endurance events. However, the same authors call attention to the fact that association studies in genetics present lim-

itations and the interpretation of the association of a single gene with a given phenotype should be carefully carried out.

In addition to its structural function in the muscular contractile equipment, the sarcomeric α -actins are also involved with metabolism-regulator proteins and signaling ways, such as fructose 1,6 bisphosphate and glycogen phosphorylase⁽⁶⁾.

C34T POLYMORPHISM OF THE AMP DEAMINASE GENE (AMPD1)

During intense and short muscular contractions, the sudden demand of ATP exceeds the capacity of the cell of its resynthesis. The ATP depletion in this situation, may reach approximate values of 40%⁽¹⁶⁻¹⁷⁾. The consequent increase of ADP (drop in the ATP/ADP ratio) in intense contractile activity, that is, an inhibiting factor of the contractile process⁽¹⁸⁾ and characteristic component of muscular fatigue⁽¹⁹⁾, is antagonist of biochemical ways, mediated by enzymes with kinase and deaminase activity. In a trial to keep the cell's energetic needs, the reaction catalyzed by the AMP deaminase (AMP \rightarrow IMP + NH₃) indirectly minimizes the ADP accumulation for removal of the AMP and dislocates the reaction balance of the adenylate kinase (2ADP \rightarrow ATP + AMP)⁽¹⁸⁾. This reaction catalyzed by the AMP deaminase and activated during intense metabolic activity in the skeletal muscle is measured by the isoform M (myoadenylate deaminase) coded by the AMPD1 gene, located in the 1 p13-p21 chromosome⁽²⁰⁾. This isoform corresponds to more than 95% of the total AMPD⁽²¹⁾ and is mainly present in type II muscular fibers⁽²²⁾. A nonsense mutation, transition of the nucleotide C \rightarrow T in the 34 position of the exon 2 of the AMPD1 gene, converts the CAA codon (glutamine) into a stop codon (TAA), resulting in an early interruption of the protein synthesis⁽²³⁾. Consequently, individuals who present the polypeptidic mutant sequence, TT homozygote or CT heterozygote, respectively, present lower and intermediate enzymatic activity of myoadenylate deaminase, when compared with the CC homozygote individuals⁽²⁴⁾. According to some authors⁽²⁵⁻²⁶⁾, part of the population which expresses the mutant gene (2% of the Caucasian population is homozygote and approximately 20% is heterozygote) is vulnerable to muscular cramps, pain and premature fatigue during exercises.

Generally speaking, the reasoning for the reduced capacity to exercise connected with myoadenylate deaminase deficiency would be grounded on the remarkable accumulation of ADP and AMP during exercise⁽¹⁸⁾. In order to test such hypothesis, some investigators have used a test with short duration and high intensity exercises (Wingate) in 18 individuals with different genotypes for AMPD1. The Wingate test, a 30-second anaerobic power test, induces expressive AMP deaminase activation⁽²⁷⁻²⁸⁾. No difference in the power peak and mean power generated during the 30-second test was identified in the different genotypes. However, despite the distribution of the fiber types among the CC (*wild-type*), CT and TT (mutant) genotypes being similar (51%, 48% and 62% of type I fibers, respectively) a remarkable difference in the AMP deaminase enzyme activity was verified, with values ranging from 1010-2169 mmol/Kg dry tissue/min. for CC, 337-632 mmol/Kg dry tissue/min. for CT and 4-14 mmol/Kg dry tissue/min. for TT. Homozygotes for the mutant gene have AMP deaminase activity lower than 1% of the enzymatic activity found in the *wild-type* individuals. Coherent with the low AMP deaminase activity found in TT individuals, no increase in ammonia levels (NH₃) post exercise, neither IMP accumulation was verified. Parallel to this, an increase of 25 times in the adenosine content in the muscle of TT individuals after exercise as well as a slight increase of two times in CT individuals were found. Adenosine is a metabolite derived from the AMP dephosphorylation by the nucleotidase 5' cytosolic enzyme and vasodilation mediator, playing an important role in the coronary blood flow regulation and lower importance in the muscular blood flow regulation⁽²⁹⁻³⁰⁾. The 5' nucleotidase enzyme is abun-

dant in the cardiac and skeletal myofibril muscles and, in this study, dephosphorylated more AMP in adenosine in the presence of the mutant genotype due to the reduced AMP deaminase activity. This reduced flow through the AMP deaminase way as well as consequent increase of 25 and 2 times in the muscular content of adenosine in the TT and CT genotypes, respectively, suggests an increase in the local blood flow, which can favor the local oxidative metabolism. Greater accumulation of AMP can also activate kinase proteins activated by AMP, which seems to increase the oxidation of fatty acids as well as the transportation of blood glucose to the muscles⁽³¹⁾.

Rico-Sanz *et al.*⁽³²⁾, when searching for an association between C34T polymorphism of AMPD1 and cardiorespiratory and performance phenotypes, submitted sedentary individuals to a physical training program for 20 weeks. These authors verified that: 1) before the training beginning, the TT genotype individuals presented higher effort exertion values (Borg scale), compared with CT and TT genotypes individuals ($P = 0.0002$). Moreover, the 50 W absolute load represented a relative exercise intensity 7% higher in TT individuals when compared with CT and CC individuals; 2) after the training period, the maximal minute ventilation, $\dot{V}O_2$ max. and $\dot{V}CO_2$ max values were lower in the TT individuals ($P = 0.01$). These outcomes suggest that homozygote individuals present reduced exercise capacity when sedentary for the mutant allele and lower ventilatory adaptation in response to training.

Rubio *et al.*⁽³³⁾ were the first ones to verify the distribution of the C34T polymorphism of the AMPD1 gene in endurance elite athletes (cyclists and runners). These authors observed a 4.8% frequency of the T allele in these athletes, while in non-athlete healthy individuals the frequency was of 8.5% (~50% higher). Among athletes, no difference in the $\dot{V}O_2$ max, ventilatory threshold and respiratory compensation point was verified in the different genotypes. Although some authors⁽³⁴⁻³⁶⁾ have verified that the AMP accumulation may also occur during submaximal exercise prolonged until exhaustion (e.g. 70-75% of $\dot{V}O_2$ max.), particularly in the presence of low muscular glycogen reserve, a potential study limitation would be that these athletes are not set in short duration and supramaximal intensity (e.g. > 110% of $\dot{V}O_2$ max.).

I/D POLYMORPHISM OF THE ANGIOTENSIN-CONVERTING ENZYME (ACE)

The endocrine renin-angiotensin system (RAS) plays an important role in the control and homeostasis of the human circulatory system⁽³⁷⁾. Renin which is produced by juxtaglomerular renal cells, a modified type of straight muscular cell located in the afferent arterioles, acts over the angiotensin globulin, releasing a 10-amino acid peptide called angiotensin I. This peptide has mild vasoconstrictor properties; however, when cleaved in a peptide of 8 amino acids, angiotensin II (Ang II), through the action of the angiotensin-converting enzyme (ACE), acquires a very relevant vasoconstrictor capacity. Such physiological response is predominantly mediated by action in specific receptors for Ang II (AT_1 and AT_2) located on the cellular surface⁽³⁾. Besides its vasoconstricting action, the Ang II provokes increase of blood pressure by the retention of salts and water in the renal tubules, secondary to the aldosterone action released by the supra-renal^(3,37). The existence of SRA in the cardiac⁽³⁸⁻³⁹⁾, adipose⁽⁴⁰⁾ and skeletal muscular tissues has also been reported⁽⁴¹⁾. Another function which determines ACE relies on the bradykinin hydrolysis by the removal of a peptide of the C terminal region⁽⁴²⁾, which causes its deactivation. Bradykinin is a peptide of vasodilation action and inhibitor of the cellular growth. In addition, it promotes its effect by action in specific B_1R and B_2R receptors⁽⁴³⁾.

The ACE gene (21 Kbp) is located in the chromosome 17 q23 and is composed of 26 exons⁽⁴²⁾. A common genetic variant in the ACE gene was described and consists in the absence (deletion or allele D) or presence (insertion or allele I) of 287 base pairs in the

intron 16⁽²⁾. The allele D is associated with increased circulatory and tissue levels of ACE⁽⁴⁴⁻⁴⁵⁾. The I/D ACE polymorphism has attracted considerable attention concerning its association with human physical performance. Recent studies demonstrated that the allele I is more frequent in endurance athletes, while the allele D in strength and muscular explosion athletes^(37,46).

In the heart, the Ang II is a potent factor of cellular growth⁽⁴⁷⁾. Although increase in the left ventricular mass has not been verified in individuals with different genotypes for ACE⁽⁴⁸⁻⁴⁹⁾, the local RAS activation with consequent increase of the Ang II in response to mechanical overload induced by physical exercise, seems to increase the protein synthesis in the cardiac myocyte via AT_1 receptors⁽⁵⁰⁻⁵¹⁾. Hypertrophy in the left ventricle (LV) is a remarkable characteristic in elite athletes⁽⁵²⁾. Nevertheless, varied levels of LV hypertrophy have been verified in endurance athletes submitted to similar training regimen, suggesting thus, that this adaptation is genetically mediated. A distribution of 44%, 51% and 5% was found for DD, DI and II genotypes, respectively in endurance athletes (male sex)⁽⁵³⁾. Moreover, athletes with DD genotype showed significantly higher values of left ventricular mass index when compared with ID genotype athletes. Interestingly enough, 70.4% of the DD athletes, 42% of the DI athletes and 0% of the II athletes reached the criteria for LV hypertrophy characterization. It is also worth mentioning that the local RAS mechanism activation by physical exercise is exacerbated in homozygote individuals for the D allele, resulting in greater bradykinin degradation. According to what has been previously pointed out, bradykinin has antiproliferative and growth inhibiting effect⁽⁵⁰⁾. Therefore, greater degradation of bradykinin may facilitate the LV hypertrophy. Yet, these outcomes do not lead us to the conclusion that I/D ACE polymorphism is the only mediator of the LV development.

With the purpose to clarify the Ang II role in the association between I/D ACE polymorphism and LV mass, Myerson *et al.*⁽³⁹⁾ submitted 141 recruits from the British Army distributed in 79 DD (38 losartan and 41 placebo) and 62 II (28 losartan and 34 placebo) to a period of 10 training weeks as well as treatment with 25 mg/day of losartan or placebo. After the experimental period, it was seen that losartan does not influence in the LV mass, with the recruits from the losartan group presenting hypertrophic response similar to their respective placebo groups. However, the recruits with DD genotype presented LV mass significantly higher in the post-training period when compared with the pre-training one. It is interesting that these outcomes persisted when the LV mass was corrected by the increase verified in the lean body weight, suggesting hence an excessive cardiac hypertrophy. The authors confirmed the I/D ACE polymorphism effect in the cardiac hypertrophy induced by physical training. They also attributed the verified outcome in the losartan group to the possible interaction of Ang II with other subtype of AT receptor.

One could wonder at this moment how the ACE genetic variant could influence the biological and mechanical properties of the tissues. The existence of a complete RAS in the skeletal muscles with potential to influence the total body energetic balance has been reported^(41,54).

Williams *et al.*⁽⁵⁵⁾ studied the muscular contractile efficiency evaluated in cycle ergometer in 58 healthy individuals (males) (35 II and 23 DD), before and after 11 weeks of a physical training program. The energy used per power unit, 'efficiency delta' (% of alteration in the work performed per minute/% of alteration in the energy spent per minute) did not demonstrate to be different between II and DD genotypes (24.5% and 24.9%, respectively) in the pre-training period. However, in response to training, this variable increased significantly among the individuals with genotype II. This difference represents an increase in efficiency related with the pre-training period, of 8.62% for the genotype II and -0.39% for the DD genotype. The authors do not know the mechanisms through which the I allele would be boosting the mechanical effi-

ciency in trained individuals. Nevertheless, they have based their findings in two possible explanations: 1) low ACE enzymatic activity in II genotype could improve the contractile function in the cardiac and skeletal muscles via improvement in the efficiency of the mitochondrial oxidation, a factor mediated by the local increase in the nitric oxide concentration⁽⁵⁶⁾ and, 2) greater muscular efficiency could be related to the constitution of the muscular fibers, with the II genotype presenting greater percentage of type I fibers (slow contraction fibers) which are more efficient than the fast contraction fibers (type II) when the muscular contractile activity is performed at low velocity.

Later, Zhang *et al.*⁽⁵⁷⁾ confirmed one of the hypotheses by Williams *et al.*⁽⁵⁵⁾. The authors verified after biopsies of the lateral vastus muscle of 41 sedentary individuals, an association between the ACE genotypes and the percentage distribution of fiber I, IIa and IIb. Individuals with II genotype when compared with the DD genotype presented higher mean percentage of type I fibers (~50% vs. ~30%, respectively) and lower mean percentage of type IIb fibers (~16% vs. ~32%, respectively). There was no difference between genotypes for the values of mean percentage for type IIa fibers. Although the mechanism through which the ACE gene determines the distribution of the different types of fiber in the muscle is not clear yet, these outcomes corroborate the studies which showed association of the I allele and high performance in endurance athletes.

Myerson *et al.*⁽³⁷⁾, have observed higher I allele frequency among long distance elite runners when compared with healthy sedentary individuals. More interestingly, the analysis of 91 Olympic level runners revealed a linear and growing tendency in the I allele frequency 0.35 0.53 and 0.62 in the specialties ≤ 200 m (n = 20), 400-3000 m (n = 37) and ≥ 5000 m (n = 34), respectively. The presence of this allele is more frequent as the distance of the completed event grows. This fact suggests that the endurance athletes' performance is, at least partly, dependent on its presence.

Hagberg *et al.*⁽⁴⁶⁾ while testing the hypothesis that the $\dot{V}O_2\text{max}$ could be affected by the I/D ACE polymorphism, evaluated 58 physically active sedentary women as well as postmenopausal athletes. The allele distribution did not differ between groups. When they were analyzed together, the genotypes frequency was: 21% II (n = 12), 57% ID (n = 33) and 22% DD (n = 13). In the three groups together, II genotype presented a $\dot{V}O_2\text{max}$ 6.3 ml/Kg/min (23%) higher than DD genotype and 3.3 ml/Kg/min (11%) higher than ID. Contrary to the expectation, the difference in the $\dot{V}O_2\text{max}$ between genotypes could not be explained by differences in the systolic volume and cardiac debt. The values for these variables were similar among groups; however, the highest values of $\dot{V}O_2\text{max}$ in the presence of the I allele were derived from the highest maximal difference a- vO_2 (II = 16.5 ± 2.0 ml/dl; ID = 15.4 ± 1.6 ml/dl and DD = 14.4 ± 1.2 ml/dl).

In summary, the data presented suggest that the I allele improves performance in endurance athletes, a fact which is mediated by the better mechanical efficiency of the skeletal muscles as well as its effect in the proportion of muscular fibers, while the D allele showed relationship with the strength and muscular explosion phenotype, which was mediated by the muscular hypertrophic effect, secondary to the increase in the plasma and tissue concentration of Ang II.

-9/+9 POLYMORPHISM OF THE BRADYKININ β_2 RECEPTOR (BDKRB2)

The angiotensin-converting enzyme (ACE) is responsible for the genesis of the vasoconstrictor substance angiotensin II and also by the bradykinin degradation⁽³⁷⁻³⁸⁾. If the ACE plays an important role in the bradykinin metabolism, it would be reasonable to associate that the its levels present an inverse relationship with the I/D ACE polymorphism, that is, high concentrations of ACE (associat-

ed with the D allele) are linked with reduced levels of bradykinin, while low concentrations of ACE (associated with the I allele) are linked with high levels of bradykinin. To put it shortly, the bradykinin levels are dependent on the ACE genotypes and can influence both glucose pick up and muscular blood flow⁽⁵⁸⁾, while it prevents the growth of the left ventricle (LV) via activation of the receptors β_2 (B_2R) for bradykinin^(43,59).

A variant in the exon 1 was found for the β_2 receptor gene (B_2R) BDKRB2, located in the chromosome 14 q32.1-q32.2, in which the absence (-9) and not the presence (+9) of a segment of 9 base pairs is associated with the high transcriptional activity of the gene and consequently, high response of the receptor to the agonist⁽⁹⁾. Therefore, if bradykinin can modulate the hypertrophic response of the LV, it is expected that the different genotypes of the BDKRB2 gene would have potential to alter the magnitude of this growth.

In order to test such hypothesis, Brull *et al.*⁽⁵⁹⁾ submitted 109 recruits of the British Army to a 10-week training period and verified the effects of the -9/+9 polymorphisms of the BDKRB2 gene and I/D ACE in the LV hypertrophy. There was a trend in hypertrophic response mean to training to be lower in the II individuals compared with the DD individuals for the ACE gene (+6.9 g vs. +11.2 g respectively; p = 0.09). Nevertheless, for the -9/-9 (n = 16), -9/+9 (n = 60) and +9/+9 (n = 33) genotypes of the BDKRB2 gene, the mean of the hypertrophy response to training was: 4.6 g vs. 8.3 g vs. 13.7 g, respectively; (p = 0.009). The hypertrophic response associated with both genotypes revealed an interesting fact. In individuals with low concentration of bradykinin and low transcriptional activity of the B_2R receptor (DD and +9/+9 genotypes) the alteration in the LV mass was of 9.5%, while in individuals with high concentration of bradykinin and high transcriptional activity of the B_2R receptor (II and -9/-9 genotypes), the alteration in the LV mass was of -0.4%. The authors suggest that, alterations in the bradykinin concentration (determined by the ACE genotype) and in the transcriptional level of the B_2R receptor (determined by the BDKRB2 genotype) biologically interact for the determination of the physiological response. The ACE effect in the LV hypertrophy seems to be mediated, at least partly, by bradykinin⁽⁹⁾.

Williams *et al.*⁽⁴³⁾ while believing that part of ACE effects in the muscular contractile function may be mediated by bradykinin, studied in two distinct populations (115 healthy individuals and 81 British Olympic athletes) the muscular contractile efficiency for the different genotypes of the BDKRB2 gene, verified by the energy used per the 'efficiency delta' power unit (% of alteration in the work performed per minute/% of alteration in the energy spent per minute) and evaluated in cycle ergometer. For the 115 healthy individuals, the efficiency delta showed association with the +9/+9, +9/-9 and -9/-9 genotypes (23.84 ± 2.41 vs. 24.25 ± 2.81 vs. $26.05 \pm 2.26\%$ respectively; p = 0.002). It is worth mentioning that the same authors⁽⁵⁵⁾ have previously shown (study presented in the previous section) that there was no association between the efficiency delta and the I/D ACE genotype, a fact that led the group to verify the existence of a biological interaction between the I/D ACE polymorphism and the -9/+9 of BDKRB2. As a result, among the DD individuals, the efficiency delta showed a tendency to be higher in the -9/-9 homozygote individuals. Nonetheless, the -9/-9 genotype of BDKRB2 influenced the efficiency delta in the individuals with II genotype for ACE (24.34 ± 2.51 vs. 24.26 ± 2.41 vs. $27.41 \pm 2.61\%$ for the +9/+9 vs. +9/-9 vs. -9/-9 genotypes; p = 0.005). The efficiency delta acted extremely higher in the (II; -9/-9) genotype when compared with the (DD; +9/+9) genotype (p = 0.0007). Among Olympic Athletes, there was a trend to increase the frequency of the -9 allele as the distance of the completed event increased (0.382, 0.412 and 0.569 for the specialists in ≤ 200 m (n = 17), 400-3000 m (n = 35) and ≥ 5000 m (n = 29), respectively). In addition, a great alleles proportion was observed ('D' and '+9') in specialists in events < 5000 m and great alleles proportion

('I' and '-9') in competitors in events > 5000 m ($p = 0.003$). These data suggest that the I/D ACE polymorphism is strongly associated with functional variables of adjacent genes, aiding in the determination of the phenotype characteristic of the athlete.

Although the existence of the B_2R expression 60 and the bradykinin release by the skeletal muscles have been already described⁽⁶¹⁾, it is not precisely defined how bradykinin could affect such physical performance phenotypes. Actually, when mediated by the B_2R activation, bradykinin increases the translocation of GLUT4 for the membrane during exercise via increase of tyrosine kinase activity induced by insulin in its receptors⁽⁶²⁾. Such fact leads to a transient increase of inositol 1,4,5-triphosphate involved in the excitement-contraction joining mechanism via increase of cytoplasmatic calcium⁽⁶³⁾. The variants in the ACE and B_2R genes become potential mediators of human physical performance.

985+185/1170 POLYMORPHISM OF THE CREATINE KINASE M ENZYME GENE (CK-M)

Endurance training with sufficient intensity and duration induces increase in $\dot{V}O_{2max}$ ⁽⁶⁴⁾. However, the interindividual differences involved in the ATP regeneration process as well as other metabolic ways potentially concerned with performance in endurance events, called attention for the genes tracking with possibility to affect such phenotype.

The creatine kinase M or CK-M gene (M = muscle) is a legitimate mediator candidate of human physical performance with potential to influence the $\dot{V}O_{2max}$ as well as the response of this variable ($\Delta\dot{V}O_{2max}$) to a physical training program⁽⁶⁵⁾. The different isoforms of CK joined with the phosphate creatine (PCr), were an important metabolic buffering system in cells with dramatic fluctuations of energetic demand. In addition to this function characterized as temporal, the CK-PCr system also plays a role of spatial buffering, involved in the transportation of the phosphoryl composite of mitochondrial energy as well as glycolytic enzymes, for the hydrolysis sites of the ATP⁽⁶⁶⁾. High cellular concentration of PCr and high CK activity could thus, buffer the accumulation of ADP supporting the favorable maintenance of the ATP/ADP relationship during periods of intense metabolic activity.

The CK is an enzymatic protein which in its active presentation consists of two subunits expressed by distinct genes. The gene of the subunit M (CK-M), with 17.5 Kbp 8 exons and 7 introns, is located in chromosome 19 q13.2-q13.3⁽⁴⁾ and the gene of the subunit B (CK-B; B = brain) is located in chromosome 14 q32.3⁽⁶⁵⁾. Therefore, three dimeric isoforms are made by the hybridization of the subunits CK-M and CK-B, structuring in CK-MM and CK-BB (homodimers) and CK-MB (heterodimer). In agreement with the transportation of the high-energy phosphoryl group of the mitochondrial compartment to the hydrolysis sites of ATP (spatial function of the CK-PCr system), a third CK isoform is expressed. Located in the intermembrane space of the mitochondria, it is predominantly found in the muscular tissue, being referred as sarcomeric (Scmit-CK). Although it is expressed by a distinct gene, located in chromosome 5 q13.3, this isoform presents high degree of homology with coding sequences of cytosolic isoforms CK-MM, CK-MB and CK-BB.

All isoforms are differently expressed by different tissues. CK-MM is abundant in the skeletal muscle, keeping high ATP concentration in the head region of the myosin while the CK-MB has high activity in the cardiac muscle and lower activity in the skeletal muscle⁽⁶⁷⁾. Although CK-MM is preferably expressed in the skeletal muscle, the activity of this enzyme showed to be at least two times lower in type I muscular fibers when compared with type II ones⁽⁶⁴⁾. It is interesting that type I muscular fibers predominantly recruited in endurance activities and recognized by the predominance of oxidative enzymatic activity, present an inverse relation with the CK-MM activity. Apple and Billardelo⁽⁶⁸⁾ while concerned

with the CK genes expression, verified an increase of 40% in CK-B RNAm as well as a reduction of 42% in CK-M RNAm in the gastrocnemius muscle of rats submitted to endurance training. These data with animal models are consistent with the absence of increase of CK-MM activity in studies with humans, suggesting hence, that this isoform plays its main function in glycolytic muscular cells and is negatively related, perhaps limiting the aerobic metabolism⁽⁶⁵⁾.

A polymorphism in the CK-M gene was detected by chain polymerase reaction and DNA digestion with the endonuclease enzyme of *NcoI* restriction. The allele with site susceptible to digestion for *NcoI* was designated as 985+185 pb allele, while the allele without the site for *NcoI* restriction was designated as 1170 pb allele⁽⁶⁹⁾. This variable defined as *NcoI* is located in the region 3' of the gene⁽⁷⁰⁾.

Rivera *et al.*⁽⁶⁴⁾, while testing the hypothesis of a relationship between the CK-M 985+185/1170 polymorphism and its influence in the $\dot{V}O_2$ variable, submitted 240 individuals (80 fathers, 80 mothers and 80 children) to an endurance training program for 20 weeks. The frequency of the 985+185 and 1170 alleles was 0.3 and 0.7 respectively, for the fathers. In this same sample, the frequency of the 985+185/985+185, 985+185/1170 and 1170/1170 genotypes were 0.49, 0.44 and 0.07, respectively. When the response ($\Delta\dot{V}O_{2max}$) to the training program associated with the different CK-M genotypes was adjusted for the co variables sex, age, body weight and initial $\dot{V}O_{2max}$, significant difference was found for the 160 fathers ($p = 0.0004$) and 80 children ($p = 0.025$). The homozygotes for the rare allele (1170/1170) showed a lower $\Delta\dot{V}O_{2max}$ when compared with the homozygotes and heterozygotes for the common allele 985+185 (985+185/1170 and 985+185/985+185 genotypes). The magnitude of the difference was of at least three times lower for the 160 fathers and 1.5 times lower for the 80 children homozygote for the rare allele compared with the other two genotypes. These outcomes partly explain the heterogeneity in the $\dot{V}O_{2max}$ response to the endurance training and sustain the hypothesis of interference of the genetic component in this variable. In the sedentary state, the $\dot{V}O_{2max}$ was different only for the fathers, with the heterozygote individuals presenting higher values when compared with the homozygotes. This was the first study to show a significant association between a polymorphism and response ($\Delta\dot{V}O_{2max}$) to a training program. Nonetheless, the *NcoI* polymorphism analyzed is located in the region 3' of the gene, outside the coding region and the regulating region of the gene. For this reason, it is slightly probable that this mutation is the direct cause of the verified association⁽⁶⁴⁾, suggesting thus, that this polymorphism would serve as a marker of the genetic difference⁽⁶⁵⁾.

Although the CK-MM is generally reported as the most active isoform, studies have verified high correlation between the increase of the CK-MB activity and oxidative capacity, estimated by the increase of the synthase citrate activity in the cardiac muscle⁽⁷²⁾ and in type I skeletal muscular fibers of individuals submitted to endurance training program⁽⁷²⁾. This phenomenon is controlled at transcriptional level, with the gene of the CK-B subunit showing a consistent positive regulation, especially in oxidative muscular fibers. On the contrary, the CK-M subunit has suffered negative regulation in response to the endurance training, suggesting hence, an inverse relationship with the muscular and cardiorespiratory endurance. The increase of the CK-B subunit expression and consequently in the content of the CK-MB and CK-BB isoforms may be energetically favorable. Enzymatic kinetics studies have verified a lower constant of Michaelis-Menten (Km) per ADP of these isoforms when compared with the CK-MM isoform⁽⁷³⁾. Yet, the study by Sylven *et al.*⁽⁷⁴⁾ also showed high correlation between the increase of the Scmit-CK activity followed by the increase of the oxidative capacity as well as increase of the CK-MB activity. These outcomes suggest a specific adaptation to the endurance training, with the increase of the oxidative capacity followed by the increase

of the CK-MB activity^(72,75). These evidences support the fact that the CK-PCr system besides its classic function of providing energy in rapid contractile muscular activity conditions (anaerobic activities), is also concerned with the aerobic metabolism and consequently with endurance activities.

CONCLUSION

Scientists and exercise physiologists have been arguing for decades on this intriguing issue: what would make certain individuals surpass marks in specific modalities and which would place them above the rest of the population? It has always been believed that, favorable factors such as nutritional status, body composition as well as psychological and social circumstances should be present and, when in balance with a myriad of biological and mechanical properties, would be therefore determining specific characteristics of physical performance. Nevertheless, the optimization of all these factors would not be sufficient, taking in consideration the variability and consequently, the interindividual differences in the anatomic, physiological and biochemical adaptations, dependent on the genetic constitution of each individual.

The human genome consists of approximately thirty thousand genes. Theoretically, the genes which determine the structural and functional characteristics intrinsic to our species should present exactly the same genetic code, which is not true from the moment in which we have knowledge of the little variations in the sequence of DNA bases, described as polymorphisms or genetic variants. Such genetic diversity in interaction with specific environmental conditions determines a phenotype, which explains many variations observed in human physical performance. With the genetic sequencing techniques, the number of identification studies of variant sequences of candidate genes concerned with performance characteristics has been increasing over the last five years⁽⁵⁾. Although we have treated in this review about a small amount of the genes already described as potential candidates to influence human physical performance in specific modalities, our main aim was to present the reader with how these studies involving genetics are outlined. Therefore, we have chosen to develop elaborated description, reviewing the cellular, physiological and molecular mechanisms of the treated genes, enabling the reader to better understand how a genetic variable would be determining distinct responsiveness in different individuals.

It is important to clarify that the characterization of a phenotype is not a product of a single gene exclusively. The first evidence of the influence of genetics over human physical performance derived from studies comparing the response of physiological variables with physical training between twins and non-related individuals⁽⁷⁶⁾. As an illustration, 60-80% of the variations in skeletal muscular mass and more than 50% of the variations of the left ventricle mass are explained by genetic factors.

Although the acknowledgment that the final outcome (phenotype) represents the integration of multiple genes added to environmental factors, the identification of talents as well as the prescription of training programs which maximize the athlete's individual potential based on the characterization of genetic variables, will be able to make a revolution in the sports science.

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