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APPLICATION OF GENETIC TESTING METHODS IN SPORT

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ABSTRACT

A study to determine the frequency of occurrence of genotype polymorphism C34T AMPD1 gene to assess physical performance Uzbek athletes. AMPD1 gene polymorphism, which is involved in the energy supply of muscle activity and metabolism was analyzed among Uzbek athletes and in the control group. Based on the comparison of the distribution of genotypes and alleles rates of AMPD1 gene, the CC genotype of AMPD1 gene association with a predisposition to the high physical performance and endurance was found.

KEYWORDS: *Gene Polymorphism, DNA, Genetic Predisposition To Sport.*

INTRODUCTION

The use of molecular-genetic markers in sports science significantly increased the predictive ability of sports orientation and selection which led to the formation of a new scientific discipline - molecular genetics of sports. The central idea of which is that the individual differences in the degree of development of certain physical and mental qualities largely depend on DNA polymorphisms.

Genetic testing in sports provides assistance to teachers, coaches and sports doctors in determining the predisposition of children and adolescents to a certain type of motor activity, in rising of athletic performance through the optimization and adjustment of the training process, and in the prevention of various diseases associated with the sportsmen occupation.

In the future, everyperson will be able to get the individual genetic map - data of variations (polymorphisms) in certain parts of the genome, which are the markers of susceptibility to locomotor activity and health risk factors. Such information has great practical importance, because it allows a person to know the potential strengths and weaknesses of his/her body and helps in the selection of optimal sports specialization, as well as in the optimization of the training process, nutrition, and will significantly limit the impact of hazards on health.

Muscle adenosine monophosphate deaminase (AMPD-M) is an important regulator of muscle energy metabolism during exercise. AMPD-M is one of the integral enzymes of purine

nucleotides cycle which catalyzing the deaminization reaction and plays an important role in the metabolism of adenine nucleotides, and determines the energy potential of the cell. In resting muscle, more than 90% ofAMPD-Min sarcoplasm is inactive and unbound with myosin. During vigorous muscle contraction 50-60% of AMPD-M binds to myofibrils. At a constant level of general activity in the period of rest, the amount of bound enzyme returns to its initial level[1].

Specific for skeletal muscle AMPD-Mis encoded byAMPD1 gene, localized in the short arm of the first chromosome (1 p13.1). During the muscle biopsy it was found that about 2% of the samples had decreased activity of AMPD-M[2]. Individuals with reduced activity of AMPD-M may experience weakness, fatigue, even after the exercise with average intensity[3]. Deficiency of AMPD-M in human occurs generally because of the single nucleotide substitution of cytosine for thymine at 34th position in the second exon of AMPD1 gene, resulting in the glutamine CAA codon is transformed into a stop codon TAA (C / T gene polymorphism). The frequency of mutant T allele is 12% among people in Europe, 19% for Afro-Americans, and 0% in the Japanese population. Mutant allele homozygotes have very low concentration of AMPD-M in skeletal muscle soduring short but highly intensive exercise does not use the entire pool of adenine nucleotides, therefore there is no accumulation ofinosine monophosphate (IMP) and ammonia.

Homozygoteswith the normal allele, on the contrary, usealmost all ATP and concomitantly accumulate more IMP and NH_3. These characteristics for heterozygotes hasan average value[3, 4].

Increased formation of ADP is a consequence of reduced concentration of AMPD-M, which reduces the maximum speed of contraction and increases the time of skeletal muscles relaxation. It was found that after the high intensity training individuals who are homozygous for the mutant allele (genotype TT) or heterozygous (genotype CT) have worse aerobic indicators than those lacking the mutant allele in the genotype (CC genotype)[5]. When performing anaerobic Wingate test, carriers of the CT and TT genotypes showed the maximum capacity of 10% less than carriers of the CC genotype[2]. The first researchregarding the distribution of AMPD1 genotypes among athletes, showed a significant decrease in the frequency of mutated T allele in elite cyclists and long-distance runners as compared with the control group[6].

A genetic background to the diversity seen in the clinical progression of heart disease is well documented. Genetic variants that lead to halted or delayed disease progression are particularly interesting as they may provide a basis for new therapies. Genetic diversity in pathways involving nucleotide metabolism are particularly important due to the latter's direct links to myocardial function and metabolic regulation[7]. Several polymorphisms of the AMP deaminase 1 (*AMPD1*) gene have been described[8]. The C34T (Glu12Stop) mutation in exon 2 is by far the most common in the general population with an allele frequency of 10–14 %[9]. Lohet al.[10] were the first to describe a benefit of the C34T mutation in patients with heart disease. This study conducted in a group of 132 patients with dilated cardiomyopathy demonstrated that the probability of surviving without transplantation for more than 5 years is 8.6 times greater in patients carrying the C34T allele. Anderson et al.[11] confirmed a protective effect in ischemic heart disease demonstrating prolonged survival associated with the C34T mutation in a prospective study in 450 patients. Another study by Gastmann et al.[12] conducted in a group of 90 patients with congestive heart failure demonstrated better prognosis in patients possessing the C34T *AMPD1* mutation. Analysis of a consecutive group of 390 patients with left ventricular

dysfunction revealed better survival in C34T allele carrier patients within a subgroup with ischemic cardiac dysfunction[13]. Other independent studies demonstrated a beneficial effect of the C34T mutation on metabolic aspects related to the cardiovascular system such as a lower level of an inhibitor of plasminogen activator and soluble von Willebrand factor in patients with coronary heart disease[14]. In contrast, three studies have indicated a lack or even a deleterious effect of the C34T *AMPD1* mutation in patients with heart disease. A large population study conducted in 935 post myocardial infarction and 433 heart failure patients with long term follow-up indicated increased mortality associated with the C34T mutation within patients with a history of myocardial infarction[15]. A prospective study in 686 patients with stable congestive heart failure did not demonstrate any impact of the C34T polymorphism on tested clinical, biochemical, echocardiographic, radionuclide or exercise parameters[16]. Analysis of 161 patients undergoing coronary revascularization for clinical parameters including heart failure and cardiac death revealed lack of any impact of the C34T mutation[17]. In case of C34T polymorphism, assessment of impact on cardiovascular system could be complicated because this mutation was found to exert deleterious effects on muscle performance[18].

In this regard, the purpose of our study was to determine the rate of genotypes with C34T polymorphism of AMPD1 gene in athletes involved in football.

Materials and Methods

Blood samples for molecular genetic analysis of AMPD1 gene polymorphism were taken from 201 Uzbek athletes involved in football and 101 individuals of the control group. The venous blood from the cubital vein in the amount of 1 mlwas used as the material for DNA extraction. For the collection, storage and transportation of blood samples, vacutainers or disposable plastic tubes with 0.5 ml of anticoagulant (conservative) were used. For further processing blood samples were stored at the temperature of not more than +4 ° C.

For extraction of DNA from whole blood, *PureLink Genomic DNA Mini Kit 250* was used ("*Invitrogen*, Carlsbad, CA, USA").

AMPD1 genotyping was performed using specific oligonucleotide primers with fluorescent probes, as well as RT-PCR Kit (manufactured by *Applied Biosystems Corporation*). Ready-made amplification reagents, containing liquid inhibited "hot start" Taq DNA polymerase, deoxynucleoside triphosphates (dNTP) and magnesium chloride with final concentrations, 200 µM and 2,5 mMrespectively, and optimized buffering system for Real Time PCR were used.Into the sterile tubes0.5-1.0 µl Primer Mixwith final concentration 10 pmol/µl, 10 µl 2.5x Reaction Mix, 7 µldH_2O, and 1-2 µlof target DNA were added. Real Time PCR was performed according to standard protocol. For Real Time PCR *GeneAmp® PCR- ABI 7500 Fast Real-Time PCRSystem* with 96-well block was used. Real Time Amplification program included: pre-denaturation at 95 °C - 100 seconds;40-45 repetitions 56-60 °C - 40-50 seconds; 95 °C - 15 seconds. FAM and ROX detectors were entered into the program.

Results and Discussion

When analyzing the distribution of genotypes and alleles rates for C34T polymorphism of AMPD1 gene in the control group and among athletes, the following results were obtained: 81% of examined athletes belonged to CC genotype. Distribution of these genotypes rates

corresponded to the distribution of Hardy-Weinberg equilibrium (χ^2= 5.16; df=1; p=0.02). Distribution of genotypes observed in the control group - CC (75%), CT (23%) and TT (2%) – also obeyed Hardy-Weinberg equilibrium.

CONCLUSION

The information-analytical search of gene markers, whose polymorphisms are associated with specific cellular metabolism of athletes and might beused as predictors of competitive success, was performed. AMPD1 gene polymorphism, which is involved in the energy supply of muscle activity and metabolism, was analyzed among Uzbek athletes and in the control group. Based on the comparison of the distribution of genotypes and alleles rates of AMPD1 gene, the CC genotype of AMPD1 gene association with a predisposition to the high physical performance and endurance was found. The correlation analysis of the gene polymorphism with indicators of physical performance in athletes showed its association with CC genotype of AMPD1 gene.

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