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Article

The Single-Nucleotide Variant rs17602729 (C34T) of the *AMPD1* Gene is Associated with Athletic Qualification and Competitive Distance in Caucasian Cyclical Sports Athletes

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Abstract: All physiological processes necessary for high athletic performance, including energy production in skeletal muscles and the peculiarities of metabolic processes (phosphogenic pathway, glycolytic, aerobic) are genetically determined. The enzyme Adenosine Monophosphate Deaminase is an important regulator of skeletal muscle energy metabolism during exercise. The identification of genetic biomarkers that determine the effectiveness of ATP resynthesis is one of the priorities of sports genetics. (1) Background: To study the associations of SNV rs17602729 (C34T) allelic variants and genotypes of the *AMPD1* gene with qualification and competitive distance in Caucasian athletes of the Southern Urals. (2) Methods: 173 people of European origin who lived in the Southern Urals region took part in the study. The first group included 123 cyclical sports athletes (speed skating, running disciplines in track-and-field): SD (short distances) subgroup – 40 sprinters (mean - 22.1 ± 2.4 y.o.); MD (middle distances) subgroup – 38 athletes (mean - 20.1 ± 2.5 y.o.); subgroup LD (long distances) – 45 stayer athletes (mean - 22.6 ± 2.7 y.o.). The control group consisted of 50 healthy non–athletes (mean – 21.4 ± 2.7 y.o.). We used the Step One Real-Time PCR System (Applied Biosystems, USA) device for real-time polymerase chain reaction. (4) Conclusions: the common allele with rs17602729 of the *AMPD1* gene can be considered as a biomarker associated with short and medium competitive distances. It can help in the selection of elite athletes who require effective performance of anaerobic sports loads. The variable T allele is an unfavorable biomarker (negative predictor) for achieving the status of Honored Master of Sports and Sport Master of International Class in athletics and speed skating, regardless of the competitive distance.

Keywords: *sports genetics; candidate genes; AMPD1; single nucleotide variant; skeletal muscles; athlete.*

1. Introduction

All physiological processes necessary for high athletic performance, including energy production in skeletal muscles and the peculiarities of metabolic processes (phosphogenic pathway, glycolytic, aerobic) are genetically determined [1-3]. The enzyme Adenosine Monophosphate Deaminase (AMPD) is an important regulator of skeletal muscle energy metabolism during exercise. During intense physical exercise, ATP reserves may be depleted. The myokinase mechanism of anaerobic ATP resynthesis begins to work. It leads to the formation of inosine monophosphate (IMF) forms. AMPD shifts the myokinase reaction towards ATP production. Thus, ATP resynthesis is maintained in case of muscle fatigue [4].

The AMPK enzyme, which catalyzes the deamination reaction, plays an important role in the purine nucleotide cycle. AMPD has been shown to stabilize the energy charge near the active myosin ATPase at the ends of the A-disk, mainly in rapidly contracting myofibrils [5]. The skeletal muscle-specific isoform M of the AMPD enzyme is encoded by the *AMPD1* gene (Adenosine monophosphate deaminase 1 type). AMPD deficiency in humans may be associated with single-nucleotide substitution of cytosine (C) for thymine (T) - C34T, rs17602729 - in exon 2 of the *AMPD1* gene. As a result of this substitution (nonsense mutation), the glutamine codon is converted into a stop codon. The protein chain breaks off, and the AMPD enzyme becomes catalytically inert [6]. People with low activity of the AMPD enzyme cannot effectively perform short-term high-intensity physical exercises, which can reduce the sports pepole's athletic performance [7]. Thus, the identification of genetic biomarkers that determine the effectiveness of ATP resynthesis is one of the priorities of sports genetics.

The aim: to study the associations of the single-nucleotide variant (SNV) rs17602729 (C34T) allelic variants and genotypes of the *AMPD1* gene with qualification and competitive distance in Caucasian athletes of the Southern Urals.

2. Materials and Methods

The study was conducted within the framework of the State Assignment of the Ministry of Sports of the Russian Federation "Improvement of technologies for sports selection and selection of sports specialization in cyclic sports (track and field athletics, speed skating)" (registration number 1022060200098-2-3.3.11 dated 06.10.2023) and was approved by the local Ethics Committee of the Ural State University of Physical Culture (UralGUFC), Protocol No. 5 of 11.01.2023 All participants signed a voluntary informed consent to participate in the study. The volume of the general population and comparable groups was calculated using the Altman`s nomogram [8].

The study included 173 participants who were randomized according to the inclusion and exclusion criteria into 2 groups: the main group – 123 athletes (speed skating, cross–country athletics disciplines), including: subgroup 1 – 40 sprinter athletes (short–distance running); subgroup 2 - 38 athletes (middle-distance running) and subgroup 3 – 45 stayer athletes (long-distance running); control group - 50 non-athletes (volunteers). The observation groups were comparable in terms of sample volume, gender and age of the participants.

Criteria for inclusion in Group 1 (athletes): residents of the Southern Urals (the city of Chelyabinsk and the Chelyabinsk region, Russia); Caucasians; male; age from 17 to 21 years; experience in cyclic sports (speed skating, cross-country athletics) - 5 years or more; signed voluntary informed consent.

Criteria for inclusion in Group 2 (non-athletes): residents of the Southern Urals (the city of Chelyabinsk and the Chelyabinsk region, Russia); Caucasians; male; age from 17 to 21 years; lack of experience in sports; signed voluntary informed consent.

Exclusion criteria from the study: residents of other regions of Russia; Asians and Africans; female; age < 17 years; refusal to comply with the study protocol; acute and chronic diseases during the period of inclusion in the study or at the stages of the study.

The buccal epithelium was removed from the oral cavity using a cervical brush (cytochet). The biological samples were separated by centrifugation and stored in a refrigerator at a temperature of -18°C. Genomic DNA was extracted according to a standard protocol on the basis of the clinical diagnostic laboratory of the Scientific Research Institute of Olympic Sports (Chelyabinsk) [9]. Allelic variants and genotypes of the SNV rs17602729 (C34T) *AMPD1* gene were determined using the TagMan SNP Genotyping Assays (Applied Biosystems, USA) technique. We used the Step One Real-Time PCR System (Applied Biosystems, USA) device for real-time polymerase chain reaction.

For SNV rs17602729 of the *AMPD1* gene encoding the enzyme adenosine monophosphate deaminase type 1 (AMPD), the C allele is considered common, and the T allele is considered variable. The following designations were used to designate the variants of the genotypes of the studied SNV: common homozygous genotype CC (cytosine / cytosine); heterozygous (intermediate) genotype CT (cytosine / thymine); variable homozygous genotype TT (thymine / thymine).

Statistical processing was performed using the licensed software package ISB SPSS version 22.0 (SPSS Inc, USA). The distribution of genotypes according to the studied allelic variants was checked for compliance with the Hardy–Weinberg equilibrium (HWE). When comparing the frequencies of alleles and genotypes in pairs, the chi-square criterion (χ^2) was used. The accepted significance level corresponded to $p < 0.05$. The association of carriers of minor (variable) or major (common) alleles and genotypes of the studied candidate genes in athletes of cyclic sports with competitive distance and sports qualifications was evaluated using the odds ratio (OR, 95% confidence interval (CI).

3. Results

3.1. Baseline Clinical Characteristics

Relevant baseline clinical characteristics of participants in this study are presented in Table 1.

Table 1. Characteristics of the study participants.

General Characteristics	1 Group (Athletes) (n1 = 123)			2 Group (Non-Athletes) (n2 = 50)	p-*value*
	SD Subgroup, (n1 = 40)	MD Subgroup, (n2 = 38)	LD Subgroup, (n2 = 45)		
Age, years (M ± SE)	22.1 ± 2.4	20.1 ± 2.5	22.6 ± 2.7	21.4 ± 2.7	> 0.05
Height, cm (M ± SE)	183.0 ± 6.43	180.22± 5.66	182.76 ± 5.16	176.39 ± 6.37	> 0.05
Weight, kg (M ± SE)	77.01 ± 9.04	73.42 ± 7.32	70.54 ± 5.11	70.12 ± 9.27	> 0.05
BMI according to Quetelet, kg/m² (M ± SE)	22.92 ± 1.94	22.61 ± 2.02	21.09 ± 1.28	22.52 ± 2.30	> 0.05

Note: BMI – body mass index; SD – short distances; MD – middle distances; LD – long distances.

The SD subgroup included 40 athletes (mean age – 22.1 ± 2.4 years); MD subgroup – 38 athletes (mean age – 20.1 ± 2.5 years); LD subgroup – 45 athletes (mean age – 22.6 ± 2.7 years); control group - 50 healthy volunteers, non-athletes (mean age – 21.4 ± 2.7 years). The age of participants (athletes and non-athletes) varied from 17 to 26 years. The groups were age matched (p-value > 0.05). Participants' height varied from 166.5 cm to 197 cm for athletes, and from 163 cm to 192 cm for non-athletes. Participant weights varied from 59.6 kg to 93 kg for athletes and from 54 to 102 kg for non-athletes. BMI varied from 18.5 to 26.9 kg/m² in athletes and from 17.50 to 29.5 in non-athletes. There were no significant intergroup differences in height (p-value > 0.05), weight (p-value > 0.05) and BMI (p-value > 0.05).

3.2. Distribution of Frequencies of Genotypes and Alleles in Athletes with Different Competitive Distances and Individuals in the Control Group

No statistically significant intergroup differences were found in the frequency of the variable allele T rs17602729 of the *AMPD1* gene in athletes compared with non-athletes (14.2% vs. 19.0%, p = 0.27) (Table 2). The distribution of genotypes in the control group (non–athletes) was: CC – 66.0%; CT – 30.0%; TT – 4.0%; in the main group: CC – 73.2%; CT – 25.2%; TT - 1.6%. The distribution of alleles and genotypes in athletes and non-athletes

corresponded to HWE: the main group (athletes) – $\chi^2 = 0.10$ (p = 0.91); control group (non–athletes) - $\chi^2 = 0.10$ (p = 1.0).

Table 2. Allele frequency and genotype distribution of the single nucleotide variant rs17602729 of the *AMPD1* gene encoding the enzyme adenosine monophosphate deaminase type 1.

Alleles, Genotypes	Athletes (n = 123)	Non-Athletes (n = 50)	χ^2	p-*value*	Odds Ratio	95% Confidential Interval
C	211 (85.8 %)	81 (81.0 %)	1.23	0.27	1.41	0.76 - 2.61
T	35 (14.2 %)	19 (19.0 %)			0.71	0.38 - 1.31
CC	90 (73.2 %)	33 (66.0 %)			1.40	0.69 - 2.85
CT	31 (25.2 %)	15 (30.0 %)	1.24	0.27	0.79	0.38 - 1.63
TT	2 (1.6 %)	2 (4.0 %)			0.40	0.05 - 2.90

The common C allele was statistically significantly associated with competitive distance in sprinter athletes compared with non–athletes (OR = 1.33 [95% CI: 0.96 – 2.93]) and in middle-distance runners compared with non-athletes (OR = 2.31 [95 % CI: 0.92 – 5.83]). In addition, sprinters showed a tendency to a relationship between the carrier of a common homozygous CC genotype and competitive distance compared with non-athletes in the control group (OR = 2.28 [95% CI: 0.83 – 6.25]) (Table 3).

Table 3. Odds ratio between competitive distance and carriage of minor or major alleles and rs17602729 of the *AMPD1*.

Alleles, Genotypes	χ^2	p-*value*	Odds Ratio	95% Confidential Interval
SD Subgroup vs. Control Group				
C	0.50	0.48	**1,33**	**0.96 - 2.93**
T			0.75	0.34 - 1.66
CC			1.20	0.49 - 2.94
CT	0.53	0.47	1.0	0.40 - 2.48
TT			0.24	0.01 - 5.13
MD Subgroup vs. Control Group				
C	3.29	0.07	**2.31**	**0.92 - 5.83**
T			0.43	0.17 - 1.09
CC			2.28	0.83 - 6.25
CT	3.26	0.07	0.53	0.19 - 1.46
TT			0.25	0.01 - 5.40
LD Subgroup vs. Control Group				
C	0.05	0.83	1.08	0.52 - 2.26
T			0.92	0.44 - 1.92
CC			1.14	0.48 - 2.70
CT	0.04	0.83	0.85	0.35 - 2.08
TT			1.12	0.15 - 8.27

Note: SD – short distances; MD – middle distances; LD – long distances.

3.3. Frequency Distribution of Genotypes and Alleles in Athletes with Different sports Qualifications

Considering that the examined athletes had different sports qualifications, the frequency distribution of alleles and genotypes of the SNV rs17602729 *AMPD1* gene was additionally studied depending on their qualifications (Table 4).

Table 4. Allele frequencies and genotype distribution of rs17602729 гена *AMPD1* gene in athletes of various qualifications.

Alleles, Genotypes	Honored Master of Sports + Sport Master of International Class	Master of Sports	Candidate Mster of Sport + Category	p-*value*
SD subgroup (n = 40)				
C	7 (87.5 %)	18 (90.0 %)	43 (82.7 %)	p > 0.05
T	1 (12.5 %)	2 (10.0 %)	9 (17.3 %)	p > 0.05
CC	3 (75.0 %)	8 (80.0 %)	17 (65.4 %)	p > 0.05
CT	1 (25.0 %)	2 (20.0 %)	9 (34.6 %)	p > 0.05
TT	0 (0.0 %)	0 (0.0 %)	0 (0.0 %)	p > 0.05
MD subgroup (n = 38)				
C	4 (100.0 %)	13 (92.9 %)	36 (62.1 %)	p > 0.05
T	0 (0.0 %)	1 (7.1 %)	14 (24.1 %)	p > 0.05
CC	2 (100.0 %)	6 (85.7 %)	23 (79.3 %)	p > 0.05
CT	0 (0.0 %)	1 (14.3 %)	6 (20.7 %)	p > 0.05
TT	0 (0.0 %)	0 (0.0 %)	0 (0.0 %)	p > 0.05
LD subgroup (n = 45)				
C	7 (87.5 %)	36 (85.7 %)	31 (77.5 %)	p > 0.05
T	1 (12.5 %)	6 (14.3 %)	9 (22.5 %)	p > 0.05
CC	3 (75.0 %)	15 (71.4 %)	13 (65.0 %)	p > 0.05
CT	1 (25.0 %)	6 (28.6 %)	5 (25.0 %)	p > 0.05
TT	0 (0.0 %)	0 (0.0 %)	2 (10.0 %)	p > 0.05

Note: SD – short distances; MD – middle distances; LD – long distances.

An intragroup analysis of the genotypes and alleles distribution according to the studied SNV in sprinter athletes showed that the frequency of the widespread homozygous CC genotype increases depending on the athlete's skill level: 75.0 %; 80.0%; 65.4% for athletes with the qualification of Honored Master of Sports + Sport Master of International Class, Master of Sports and Candidate Master of Sports + Category, respectively. However, the intergroup differences did not reach statistical significance ($p > 0.05$). Similar dynamics were observed in middle- and long-distance runners.

4. Discussion

The obtained results suggest that the widespread allele with rs17602729 of the *AMPD1* gene can be considered as a potentially favorable genetic variation that increases athletic performance in cyclic sports regardless of the competitive distance. The AMPD enzyme, which catalyzes the deamination reaction of adenosine monophosphate in skeletal muscles, plays an important role in the purine nucleotide cycle. During intense physical exercise, ATP reserves may be depleted. As a result, the myokinase mechanism of anaerobic ATP resynthesis is activated, which leads to the formation of forms of inosine monophosphate. AMPD shifts the myokinase reaction towards ATP production. Thus, ATP resynthesis is maintained in case of muscle fatigue [10]. The carriage of the variable T allele is associated with the formation of a stop codon, the breakage of the protein chain and the AMPD enzyme becomes catalytically inert, which can have a negative impact on the energy supply efficiency to skeletal muscles, myopathy, increased muscle fatigue. The results of this study are consistent with the results of previous ones that established a decrease in the frequency of the variable allele T rs17602729 of the *AMPD1* gene among

high-performing athletes compared with participants in the control group (non-athletes) [4], [6], [11].

The absence of a statistically significant association of the variable T allele with athletes' sport qualifications may be due to the fact that in the process of natural selection in achieving the status of elite athletes, partial metabolic deficiency in heterozygous elite athletes, associated with a decrease in the frequency of purine nucleotide replacement, is compensated by other environmental factors. An increase in local blood flow and oxidative phosphorylation in working skeletal muscles can also be considered as possible compensating mechanisms [5].

5. Conclusions

The common allele with rs17602729 of the *AMPD1* gene can be considered as a biomarker associated with short and medium competitive distances. It can help in the selection of elite athletes who require effective performance of anaerobic sports loads. The variable T allele is an unfavorable biomarker (negative predictor) for achieving the status of Honored Master of Sports and Sport Master of International Class in athletics and speed skating, regardless of the competitive distance.

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Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the local ethics committee of the Ural State University of Physical Culture (UralSUPC), Protocol No 5 dated January 11, 2023).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

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Conflicts of Interest: The authors declare no conflict of interest.

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