ORIGINAL ARTICLE

Association of muscle-specific creatine kinase (CKM) gene polymorphism with combat athlete status in Polish and Russian cohorts

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Abstract

Background & Study Aim: Combat sports are characterized by the involvement of both aerobic and anaerobic metabolic pathways in which the effort is of intermittent characteristics and explosive type of movements are repeated over time at high intensity. The A/G polymorphism (rs8111989) of the muscle specific creatine kinase (CKM) gene, encoding the protein which plays a key role in energy homeostasis of muscle cells, has been associated with physical performance. Specifically, the CKM G allele has been reported to be linked with power athlete status, whilst the CKM A allele has been significantly over-represented in endurance athletes. The aim of our study was to investigate the association between the CKM A/G polymorphism and combat athlete status in Polish and Russian cohorts.

Material & Methods: The study was carried out in 159 combat athletes and 1512 sedentary individuals from Poland and Russia. DNA was extracted from buccal cells donated by the subjects and genotyping was carried out using PCR based methods.

Results: We found that the frequency of the CKM G allele was significantly higher in the combined cohort of Polish and Russian athletes compared with controls (41.2 vs. 35.6%, P = 0.047).

Conclusions: The results suggest that the CKM gene is associated with combat athlete status in Polish and Russian populations. Although more replication studies are needed, the preliminary data suggest an opportunity to use the analysis of CKM polymorphism along with other gene variations and standard phenotypic assessment in combat sports selection.

Keywords: CKM • gene polymorphism • combat sports • physical performance

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INTRODUCTION

Combat sports are characterized by the involvement of both aerobic and anaerobic metabolic pathways in which the effort is of intermittent characteristics and explosive type of movements are repeated over time at high intensity [1-3]. Intermittent activity is determined by high-intensity motion (with energy mostly furnished by adenosine triphosphate (ATP), creatine-phosphate and anaerobic pathways) and low intensity motion (in which the aerobic pathways have the function of active recovery).

The supply of energy for muscular activity is one of the most important factors that affect human physical performance. Consequently, the detection of genetic markers which determine the efficiency of the ATP resynthesis mechanisms is essential to modern sports biology. One of the key enzymes which play a role in energy homeostasis of muscle cells is creatine kinase (CK; EC 2.7.3.2) [4-7]. CK is mainly present in skeletal muscle, the heart, as well as the brain, and several tissue-specific isozymes [8]. One of them is muscle specific CK (CKM). This isozyme is characteristic of skeletal muscles, but the CKM activity level is significantly higher in type II (fast-twitch) than in type I fibres (slow-twitch) [9]. The protein is localized at the M line of the myofibril subfragment, one of the heavy meromyosine in the immediate proximity of the actomyosin ATPase, and the outer membrane and vesicles of the sarcoplasmic reticulum [10]. Therefore, CKM supplies the working myosin heads with ATP [8]. It also regulates the calcium ion flux during the tension and relaxation phases [11]. Additionally, the protein plays a role in the transport of energy generated by oxidative phosphorylation to muscular contraction proteins [12, 13].

The muscle specific CK is encoded by the CKM gene, which is situated on the 19th chromosome in locus 19q13.2 – 13.3. The gene extends over 17.5 kilobase pairs and includes 8 exons and 7 introns [14]. Ckm-knockout mice are viable and demonstrate no visible abnormalities. Though maximal force of their muscle is regular, they do not have the capacity to maintain maximal muscular output during short-term high-resistance work. Interestingly, they show increased aerobic performance and decreased fatigability after long periods of physical exercise [4,15]. Therefore, the reduced activity of the gene product may be a characteristic feature of the skeletal muscle of endurance athletes [5,7,16].

More than 250 polymorphic sites have been identified within the CKM gene [6]. In the context of sport research, the most analysed is the A/G DNA variation (rs8111989) in the 3'-untranslated region of the CKM gene, also known as the CKM-NcoI single-nucleotide polymorphism [7]. An association of the polymorphism with individual differences in VO_2max responses to endurance training has been demonstrated [16]. The CKM G allele has been reported to be linked with power athlete status, whilst the CKM A allele has been significantly over-represented in endurance athletes [7]. On the other hand, the CKM A/G polymorphism failed to show any significant relationship with top-level endurance capacity [17-19]. Thus, the obtained results are still unclear.

Since combat sports in terms of metabolism require both power and endurance performance, one might suggest that the CKM A/G polymorphism would be associated with the ability to become a combat athlete. Therefore, the aim of our study was to investigate the association between the CKM A/G polymorphism and combat athlete status in Polish and Russian cohorts.

MATERIALS AND METHODS

Ethics Committee

The University of St Petersburg Ethics Committee, Russia, and the Pomeranian Medical University Ethics Committee, Poland, approved the study and written informed consent was obtained from each participant. The study complied with the guidelines set out in the Declaration of Helsinki.

Subjects and controls

The study was conducted among 102 Polish combat athletes (judo, n = 29; wrestling, n = 35; boxing, n = 38) and 57 Russian combat athletes (boxing, n = 13; wrestling, n = 44). The athletes were selected based on the level of player achievements in regional and national competitions, type of discipline, and years' experience participating in sport.

Controls included 342 Polish students of the University of Szczecin and 1170 unrelated healthy Russian citizens of St Petersburg and Kazan, with no competitive sport experience. The athlete and control groups were all Caucasians.

Genetic analysis

Polish samples

The buccal cells donated by the participants were collected in Resuspension Solution (GenElute Mammalian Genomic DNA Miniprep Kit, Sigma, Germany) with the use of sterile foam-tipped applicators (Puritan, USA). DNA was extracted from the buccal cells using a GenElute Mammalian Genomic DNA Miniprep Kit (Sigma, Germany) according to the producer protocol.

The studied polymorphism (rs8111989) is an A/G DNA variation in the 3'untranslated region, formerly identified as an NcoI restriction site. All samples were genotyped in duplicate using an allelic discrimination assay on a Rotor-Gene real-time polymerase chain reaction (PCR) instrument (StepOne™, Applied Biosystems, USA) with TaqMan probes. For the discrimination of the CKM A and G alleles (rs8111989), TaqMan® Pre-Designed SNP Genotyping Assays were used (Applied Biosystems, USA) (assay ID: C_3145002_10). Genotypes were assigned using all of the data from the study simultaneously.

Russian samples

Genotyping of athletes and controls was performed with DNA samples obtained from epithelial mouth cells by alkaline extraction [20] or using DNA-sorb-A and Proba-GS sorbent kits according to the manufacturers' instructions (Central Research Institute of Epidemiology and DNA-Technology, Russia), depending on the method of sample collection (buccal swab or scrape). Samples were genotyped for the rs8111989 polymorphism by PCR and restriction enzyme digestion. PCR primers were forward 5'-GGG ATG CTC AGA CTC ACA GA-3', reverse 5'-AAC TTG AAT TTA GCC CAA CG-3', generating a fragment of 359 bp. PCR products were digested with Bsp19I restriction endonuclease (SibEnzyme, Russia) for 8 hours at 37°C and then were separated by 6% polyacrylamide gel electrophoresis, stained with ethidium bromide, and visualized in UV light.

Statistical methods

Any differences in genotype and allele frequency were analysed using $\chi 2$ tests. All calculations were performed using STATISTICA (StatSoft, Inc. 2011). STATISTICA (data analysis software system), version 10 (www.statsoft.com.), except Hardy-Weinberg equilibrium, which was examined with the programming language and environment R (http://www.r-project.org) using the exact test. P values <0.05 were considered statistically significant.

RESULTS

The frequencies of the CKM genotypes and alleles did not differ between Polish and Russian controls or athletes (data not shown), supporting our previous observations that Polish and Russian populations have similarities in their genetic profile [21-32]. Therefore, for the main analyses we used the combined data (i.e. combined groups of Caucasians, independent of precise ethnicity). The results of the distribution of CKM A/G variants in athletes versus controls are presented in Table 1.

In both athlete and control subjects, the CKM genotypes conformed to Hardy-Weinberg expectations (P = 1.000). We found that the frequency of the CKM G allele was significantly higher in the combined cohort of Polish and Russian athletes compared with controls (41.2 vs. 35.6%, P = 0.047).

DISCUSSION

Articles concerning genetic predispositions for combat sports are still limited. Moreover, the hypothesis referencing the role of the CKM gene in physical performance status has not been clearly proven. The present study is the first report regarding the A/G polymorphism (rs8111989) in the 3'-untranslated region of the CKM gene, formerly identified as a CKM-NcoI SNP, in Polish and Russian combat athletes. Our main finding was that the CKM G allele (also known as a 'power allele' [7]) was significantly higher in combat athletes compared with controls.

Combat sports are generally described as mixed power/endurance activities. The acyclic nature of these sports distinguishes them from other activities, and involves a mixture of bioenergetic pathways - competitive combat

Table 1. The CKM A/G genotype and allele frequencies in the combined (Polish and Russian) cohort of combat athletes and controls

Sport	n	Genotypes, %			P†	Alleles, %		P*
		A/A	A/G	G/G		A	G	
Judo	29	34.5	37.9	27.6	0.084	53.4	46.6	0.084
Wrestling	79	36.7	44.3	19.0	0.313	58.9	41.1	0.155
Boxing	51	37.3	49.0	13.7	0.777	61.8	38.2	0.582
All	159	36.5	44.6	18.9	0.114	58.8	41.2	0.047
Controls	1512	42.1	44.6	13.3	1.000	64.4	35.6	1.000

† - P value ($\chi 2$ test) for genotypic comparisons; * – P value ($\chi 2$ test) for allelic comparisons.

performance uses both aerobic and anaerobic energy systems [2,26]. Some authors have reported that combat athletes are more powerful than endurance athletes [1]. Judo for example, exhibits many of the characteristic features of power sports, such as high intensity, irregular exercise, and specific predispositions needed in combat sports, including power endurance, reactive power, and throwing power. However, if we consider the time of the fight (e.g. in judo it is 5 minutes for males and 4 minutes for females), the predispositions to prolonged muscle work may be examined as one of the key parameters which contributes to determining the result of the fight [2,26]. Thus, judo has been characterized as a power sport, requiring huge reserves of anaerobic power and capacity, yet working within a well-developed aerobic system [2]. Although aerobic power seems to be an important part of judo physiology, current research indicates that the VO_2 values for elite judo athletes are higher than normal, but not as high as endurance athletes [2]. Thus, the A allele may be one of the factors beneficial to endurance performance, as it leads to a prolonged capacity for muscular effort. On the other hand, the presence of the G allele contributes a greater expression of power and strength qualities [7]. In terms of the CKM gene polymorphism, our findings support the notion that power and strength play a greater role in the determination of success in combat sports.

The CKM gene, which encodes a key protein in energy homeostasis of muscle cells, was taken into consideration as a genetic marker, because the gene was shown to be associated with athletic performance and to contribute to differences in VO_2max responses following endurance training [7,16]. Previously, Rivera et al. [16] indicated that the polymorphism in the CKM gene was associated with change in VO_2max in response to endurance training. The authors analysed the polymorphism among Caucasian participants, and found significant association between the gene polymorphism and VO_2max in the sedentary state, as well as its response (ΔVO_2max) to a standardized 20-week endurance training program. The AG genotype heterozygotes showed a larger ΔVO_2max than the GG homozygotes. While there are considerable differences in the allelic and genotypic frequencies between the Chinese population and those in Europe and America, Zhou et al. [5] obtained similar results. They described a significant association between the polymorphism and individual running economy response to endurance training. The heterozygotes also showed larger steady-state consumption of oxygen, steady-state consumption of oxygen in relation to mean body weight, steady-state consumption of oxygen in relation to mean lean body weight, and ventilatory volume than those with the AA and GG genotypes. On the other hand, Döring et al. [33] genotyped 316 elite male Caucasian endurance athletes and 304 sedentary controls, finding no significant association between seven different CKM SNPs, including the studied polymorphism, and elite endurance performance status. Rivera et al. [17] also indicated that NcoI DNA sequence variant, as well as other polymorphisms, did not contribute to the proper classification of 124 Caucasian males as elite endurance athletes according to comparisons made with 115 sedentary controls. Additionally, no significant differences in the CKM genotypes and allele frequencies were found between endurance athletes and controls including 50 elite Spanish professional cyclists [18], 380 marathon runners [6], and 141 top-level rowers, runners and road cyclists [19].

Other authors [7] studied the distribution of the CKM genotypes and alleles in athletes engaged in different sports. It was established that the CKM A allele ($P < 0.0001$) and AA genotype ($P = 0.0003$) was significantly over-represented in Russian endurance athletes (biathletes, cross-country skiers, Nordic combined athletes, all-round speed skaters) compared with controls. The A allele carriage probably influences gene expression and may result in a decrease in the CKM activity in myocytes, leading to a more intense activation of oxidative phosphorylation, thereby giving priority to endurance development. On the other hand, the frequency of the CKM GG genotype ($P = 0.0001$) was significantly higher in Russian weightlifters compared to the control group [7]. Thus, it seems that the CKM A allele and AA genotype are associated with a greater predisposition to the kinds of sports mainly aimed at endurance development. Whereas, higher frequency of the G allele in weightlifters suggests that it contributes to a greater expression of power and strength qualities [7].

It is very interesting, yet unclear how CKM A/G polymorphism may affect gene regulation and protein expression. Probably, its localization in the 3'-untraslated region is the direct cause of the association with some phenotypes [16]. This polymorphism might also influence the intracellular localization of its mRNA, and affect its stability and the rate of transcription, leading to differences in its expression [34]. A potential association has been described between the CKM gene and susceptibility to exertional rhabdomyolysis (ERB), a clinical syndrome of skeletal muscle destruction in response to exercise. People with the AA genotype have a six-fold higher risk compared with those with the GG and AG genotypes for exhibiting an exaggerated CK response to exercise. Thus, the G allele may be associated with a protective mechanism against exertional muscle breakdown [35].

Unfortunately, none of the aforementioned authors examined combat sports athletes, so the obtained results of the present study cannot be compared. These conclusions should be supported with more experimental studies involving combat sport athletes, larger populations and differing ethnicities. What is more, other CKM polymorphisms and other such genes which may describe a common phenotype among elite combat athletes should be taken under consideration.

In conclusion, the rs8111989 A/G variation in the 3'-untranslated region of the CKM gene is associated with combat athlete status in Polish and Russian cohorts. Although more replication studies are needed, the preliminary data suggest an opportunity to use the analysis of CKM polymorphism along with other gene variations and standard phenotypic assessment in combat sports selection.

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