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A single nucleotide polymorphism rs553668 in the *ADRA2A* gene and the status of Polish elite endurance athletes

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Introduction. The variance in human athletic ability is the result of interaction of both genetic and environmental factors. The *ADRA2A* gene that encodes adrenergic receptors α2 is likely to be a candidate gene because ADRA2A receptors are crucial for precise cardiovascular control and are involved in the regulation of adipocyte lipolysis, glucose metabolism and insulin secretion. Several genetic variants of the *ADRA2A* gene have been identified, and one nucleotide polymorphism (SNP) rs553668 seems to be of special importance. On the basis of results of available studies it is assumed that the C allele of rs553668 might be associated with the status of Polish elite endurance athletes. Aim of the Study. The purposes of the study were to determine the distribution of the *ADRA2A* rs553668 SNP genotypes within a sample of Polish elite endurance athletes and sedentary controls to investigate a possible association between genetic polymorphisms in the *ADRA2A* gene and elite endurance athlete status and to check for an association between the rs553668 genotypes and alleles and the athlete status. Material and Methods. The study was performed on a group of 123 elite Polish endurance-oriented athletes. Control samples were prepared from 228 unrelated, sedentary volunteers. Results. No statistical differences were found between the endurance athletes and the control group across the *ADRA2A* C/T genotypes. Similarly, no statistical differences among the subgroups of top-elite, elite and sub-elite endurance athletes were observed. Conclusions. We found that the C allele as well as C-containing genotypes were not significantly more frequent in endurance athletes than in controls. This may suggest that harboring the T allele of the SNP rs553668 allele does not decrease the probability of being an endurance-oriented athlete in the Polish population. In respect to the analyzed population of Polish endurance athletes the *ADRA2A* gene can not be considered a candidate determinant of individual variations in exercise-related phenotypes.

KEY WORDS: *ADRA2A* gene, rs553668, polymorphism, elite athletes.

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What is already known on this topic?
ADRA2A receptors are crucial for precise control of cardiovascular function as well as are involved in the regulation of adipocyte lipolysis and glucose metabolism. Some of the allelic variants of *ADRA2A* gene have distinct functional effects on receptor expression/function, therefore *ADRA2A* gene variability could favour aerobic performance. No data are available on Polish endurance-oriented athletes.

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Introduction

Endurance performance triggers an extraordinary enhancement in the aerobic phenotype and is influenced by numerous extrinsic and intrinsic factors. There are widely known extrinsic factors like training and nutrition that have an obvious effect on athletes. The intrin-

sic components encompass, for example, age, gender, physiological, biochemical and even psychological characteristics that depend more or less on the genetic background. The variance in human athletic ability is therefore the result of interaction of both groups of factors also referred to as nurture (environment) vs. nature (genetics) [1]. The DNA sequence made up of a combination of four chemical bases is localized in mitochondria and the nucleus of the cells, and it determines the biological information necessary to form all tissues of the human body. The sequences referred to as genes that produce biological molecules are, for example, responsible for optimal cellular metabolism as well as cardiovascular and cardiorespiratory functions. At least 50 genes or DNA sequences have been associated with endurance performance in literature [2]. Among them the adrenergic receptor (ADR) genes are of special interest because the products of these genes being members of G protein-coupled receptors mediate many biological functions of catecholamines such as epinephrine and norepinephrine [3]. ADRs are, therefore, crucial for precise control of cardiovascular parameters such as heart rate, stroke volume, cardiac output and mean arterial blood pressure and are involved in the regulation of adipocyte lipolysis, glucose metabolism and insulin secretion [4]. The ADR genes are classified as alpha (ADRA) and beta (ADRB). The *ADRA2A* gene, which is a member of the ADRA gene group, consists of an intronless, single 3650-base pair exon located on chromosome 10q24–q26 [5]. The gene encodes the ADRA2A receptor that modulates cardiovascular function by way of central inhibition of sympathetic activity or feedback inhibition of norepinephrine release from sympathetic neurons [6]. When activated, these receptors induce bradycardia, hypotension and sedation. In the aspect of lipid metabolism activated ADRA2A receptors restrain adipocyte lipolysis by inhibition of hormone-sensitive lipase [7]. Several genetic variants of the *ADRA2A* gene have been identified, with some having distinct functional effects on receptor expression or function [4, 8].

Small et al. [4] found that the T allele of the single nucleotide polymorphism (SNP) rs553668 resulted in an increased expression of the *ADRA2A* gene. Clinical genetic association studies revealed that the rs553668 T allele was associated with hypertension [9], fasting serum glucose values and diabetes, [10] and that the carriers of T allele reduced insulin secretion and elevate mRNA expression, which could increase the risk of type 2 diabetes [11]. A higher expression of the T allele of *ADRA2A* gene may also lead to restricted lypolysis and then to obesity. By contrast it might be assumed that the C allele is associated with the normal expression of the *ADRA2A* gene and is responsible for the proper *ADRA2A* mRNA level, which, for instance, could be beneficial for endurance athletes.

Indeed, Wolfarth et al. [12] suggested that the C allelic version rs553668 of the *ADRA2A* gene may play a certain role in sustaining the endurance training regime to attain a high level of maximal aerobic power in elite endurance athletes. That is why, in our opinion, the *ADRA2* gene is likely to be a candidate gene of aerobic performance and trainability. Taken together, the accumulated data suggest that DNA sequence variants within the *ADRA2A* gene could lead to different control of cardiovascular function, lipid and glucose metabolism in athletes, and that the C allele of SNP rs553668 might be associated with the elite endurance status of Polish athletes. The aim of the study was to determine the distribution of the *ADRA2A* rs553668 SNP genotypes within a group of Polish endurance athletes and sedentary controls in order to investigate the possible association between genetic polymorphisms in the *ADRA2A* gene and the elite endurance athlete status, and to check for an association between the rs553668 genotypes and alleles and athlete status.

Materials and Methods

Athletes and controls

The experimental procedures were prepared in accordance with the set of principles for reporting results of genetic association studies defined by the STrengthening the REporting of Genetic Association Studies (STREGA) Statement [13]. The local ethics committee at the Pomeranian Medical University approved the investigation procedures used in the study. All participants gave their informed consent for the genotyping with the understanding that it was anonymous and that the obtained results would be confidential. The study was performed on a group of 123 Polish, current and former, professional endurance elite athletes (100 men and 23 women), characterized by predominantly aerobic energy production (longer that 5 minutes, with moderate to high intensity). The athlete sample size was limited because we wanted to ensure that all subjects were at the upper end of human endurance performance continuum.

The group of athletes comprised triathletes (n = 4), race walkers (n = 6), road cyclists (n = 14), 15-50 km

cross-country skiers (n = 6), marathon runners (n = 12), rowers (n = 53), 3-10 km runners (n = 17) and 800-1,500 m swimmers (n = 11).

All athletes recruited for the study were ranked in the national top 10 in their respective disciplines. The study group was stratified using the classification by Druzhevskaya et al. [14]. It included 28 athletes classified as 'top-elite' (gold medalists in the World and European Championships, World Cups or Olympic Games) and 60 athletes classified as 'elite' (silver or bronze medalists in the World and European Championships, World Cups or Olympic Games). The others (n = 35) were classified as 'sub-elite' (participants in international competitions). Various methods were used to obtain the samples, including targeting national teams and gaining information from the national coaching staff and athletes attending training camps.

Control samples were prepared from 228 unrelated, sedentary volunteers (students of the University of Szczecin, aged 19-23; including 58 females and 170 males). All athletes and controls were Caucasian in order to reduce the possibility of racial gene skewing and to overcome any potential problems due to population stratification.

Molecular analyses

All molecular analyses were performed in the Department of Genetics, Faculty of Biology of the University of Szczecin. Genomic DNA was extracted from the buccal cells donated by the participants using a GenElute Mammalian Genomic DNA Miniprep Kit (Sigma, USA) according to the manufacturer's protocol. All samples were genotyped using an allelic discrimination assay on a Rotor-Gene real-time polymerase chain reaction (PCR) instrument (Corbett, Australia) with TaqMan probes. For the discrimination between the *ADRA2A* C and T alleles (rs553668) a TaqMan Pre-Designed SNP Genotyping Assays were used (Life Technologies, USA), including primers and fluorescently labelled (FAM and VIC) MGB probes for the detection of the alleles.

Statistical analysis

The STATISTICA software package, version 8.0, was used for all statistical calculations. Allele frequencies were determined by gene count. A $\chi 2$ two-sided test with Yates correction was used to compare the *ADRA2A* C and T alleles and the genotype frequencies between the athletes and control participants as well between athletes at different competitive levels. The level of statistical significance was set at $p < 0.05$.

Results

The *ADRA2A* C/T genotype distribution was in agreement with the Hardy–Weinberg equilibrium within the athlete and the control group (both groups tested separately, $p < 0.05$). There were no statistical differences between the endurance athletes and the control group across the *ADRA2A* C/T genotypes, as shown in Table 1. Similarly, no statistical differences among the subgroups of top-elite, elite and sub-elite endurance athletes were observed (Table 1).

What this paper adds?

To our knowledge this is the first report comparing the allele as well as genotypes distribution of single nucleotide polymorphism rs553668 within *ADRA2A* gene between non-athletic controls and Polish endurance-oriented elite athletes. The results from the present study should facilitate understanding the possible contribution of genetic variants to the complex phenotype of professional athletes what is currently the matter of investigation worldwide. We hope that this manuscript encourage other scientist from Poland as well as other countries to undertake the work required to establish whether genes' variants with known functional roles in human physiology influence human physical fitness. Once the polymorphisms related to athletes performance are described, it may be possible to develop genetic tests hat may be used to identify sport talent.

Discussion

It has been demonstrated that genes may influence athletic ability to a high degree, and a significant heritability (which refers to how phenotypic variance is attributable to genetic effects) has been observed for traits relevant to athletic performance (e.g. aerobic capacity [15, 16], cardiovascular parameters [18], energy expenditure and substrate utilization [17, 19]). Despite the fact that these observations provide evidence of genetic involvement in creating sports abilities, they do not reveal what specific genes or DNA sequences are behind this process.

The last two decades have brought advances of several molecular methods that have been successfully used to investigate the human genome. That knowledge enabled scientists representing different fields to study genetic contribution to many complex traits including exercise

science and sports medicine. The importance of allelic variants of candidate genes and their associations with the athlete status as well as performance have been widely discussed in recent years (20-23). The present study reports a genetic case-control association, which is an approach widely applied to identify specific candidate genes. The genes are considered candidates because of their known function presumed to be relevant to the trait under study [1]. For that reason we compared the frequencies of alleles as well as genotypic distribution of the SNP polymorphism rs553668 within the *ADRA2A* gene between athletes of the highest possible endurance level in Poland and non-athletic controls being a random representation of the general population. The main finding of the study is that the C allele as well as C-containing genotypes were not significantly more frequent among the endurance athletes than among the controls. Thus, these findings do not support the hypothesis that variation in the *ADRA2A* gene locus has an influence on control of cardiovascular function as well as glucose and lipid metabolism in Polish endurance athletes. They also suggest that harboring the T allele of the SNP rs553668 does not decrease the probability of being an endurance-oriented athlete in the Polish population. Our results are therefore in opposition to those of Wolfarth et al. [12] who found evidence of a weak association between the C allele of the rs553668 polymorphism and endurance performance status.

A number of issues must be highlighted to explain the differences between our results and the results of Wolfarth et al. [12]. The first is that there are few other polymorphisms and mutations of relevance within the *ADRA2A* gene [24]. Thus, it can not be excluded that other allelic variants in the gene alone or in the haplotype combination could be of importance, and that not possessing the CC genotype, as it could be expected for the endurance athlete, does not necessarily limit sports performance as it might be compensated for by other allelic variants in the *ADRA2A* gene, as seen in the *ADRB2* gene [22].

The second problem is that the endurance performance phenotypes are highly polygenic. Based on mathematical calculations of Williams and Folland [20] it has been estimated that there is just a 0.0005% probability that even a single individual exists in the world possessing the optimal DNA variant for endurance performance of all 23 "endurance" polymorphisms analyzed by the authors. In other words, there would be an expectation to find a few individuals with selected 16 of the 23 optimal genotypes, but probably no individuals with 17 genotypes in the world population. Therefore, it is extremely unlikely that elite endurance athletes possess all or nearly all of the favorable allelic variants [20]. In our previous studies on the beta-adrenergic receptor 1 and 2 (*ADRB1* and *ADRB2*) genes we demonstrated that the allelic version of 49Gly allele and haplotype combination of 49Gly:Arg389 allelic of the *ADRB1* gene were associated with the status of Polish elite endurance athlete [25]. However, the *ADRB2* gene Gly16Arg and Glu27Gln markers analyzed by Williams and Folland were not associated with the endurance phenotype [26].

The third issue that needs to be emphasized is the ethnicity of the population under study. It has been shown that if a significant association with physical performance is observed in one population for some polymorphic variants, not necessarily the same association or even lack of association is noticed in another population [27]. Because the Wolfarth et al. [12] examined a Caucasian population, however of different ethnic background than the population analyzed in this study, we can not

Table 1. Genotype distribution and allele frequencies of *ADRA2A* gene rs553668 polymorphic site in Polish athletes and control group

Group	n	*ADRA2A* C/T genotype			p	C allele %	p
		CC (%)	CT (%)	TT (%)			
Endurance-oriented	123	**90 (73.2)**	**31 (25.2)**	**2 (1.6)**	**0.775**	**85.8**	**0.378**
top-elite	28	21 (75.0)	7 (25.0)	0 (0.0)	0.916	87.5	0.494
elite	60	44 (73.3)	15 (25.0)	1 (1.7)	0.918	85.8	0.527
sub-elite	35	25 (71.4)	10 (28.6)	1 (2.9)	0.904	85.7	0.677
Controls	228	**157 (68.9)**	**64 28.0)**	**7 (3.1)**	**1.000**	**82.9**	**1.000**

exclude the possibility that our results are distinctive to the Polish population of elite endurance athletes. Finally, it is useful to recognize that there is a very strong tendency to publish studies with positive results, and very few negative studies, in fact, become published [28]. Thus it can not be excluded that non-published data exist that shows a lack of association of the rs553668 in the *ADRA2A* gene with the athlete's performance, phenotype or status.

Our study is not without limitations and its results should be interpreted with caution because the size of our group of athletes was relatively small. However, considering the limited number of Polish elite athletes, we were unable to recruit additional samples for the study. As it was mentioned before, there might also be other genetic variants within or near the studied gene that should be further analyzed to show complex interactions. It is worth noting that the epigenetic mechanisms that change the gene function are also important when determining the elite athletic phenotype. Thus, additional large-scale prospective studies involving the interactions mentioned above are needed.

Conclusions

We found that the C allele as well as C-containing genotypes were not significantly more frequent among endurance athletes than in controls. These findings do not support the hypothesis that variation in the *ADRA2A* gene locus has an influence on the control of cardiovascular function as well as glucose and lipid metabolism in Polish endurance athletes. It is also suggested that harboring the T allele of the SNP rs553668 allele does not decrease the probability of being an endurance-oriented athlete in the Polish population. Therefore, in respect to the analyzed population of Polish endurance athletes, the *ADRA2A* gene can not be considered a candidate to understand individual variations in health- and exercise-related phenotypes.

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