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Comparison of Potential Biomarker, ACTN3 rs1815739 Polymorphism, for Athletic Performance of Turkish Athletes

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ABSTRACT

α-Actinin-3 is one of the key components of the Z-line structure of sarcomeres and also have importance in muscle cell signaling processes. Therefore, α-Actinin-3 gene rs1815739 polymorphism is one of the most analysed potential genetic biomarkers in sports genetic studies. We aimed to evaluate the genotypic and allelic distribution of α-Actinin-3 gene rs1815739 polymorphism in Turkish athletes. For this purpose, a total of 131 athletes (39 cyclists, 34 sprinters and distance athletes, 33 volleyball players, 15 bodybuilders and 10 ironmen) and 89 sedentary individuals were enrolled in the study. Genomic DNA from venous blood were isolated by using the PureLink DNA isolation kit by following the manufacturers' instructions. The α-Actinin-3 rs1815739 genotyping was carried out by Real-time PCR using the commercially provided Taqman Genotyping Assay. The statistical evaluations were assessed by the chi-square testing using the GraphPad InStat statistical package. Results showed that cyclists, ironmen and volleyball players showed statistically significant differences in terms of the genotype when compared to the controls. The OR of having the dominant trait (RR genotype vs. RX+XX combined) was 0.5 (95%CI: 0.28–0.91; P= 0.02) which was statistically significant, while the OR of having the recessive trait (XX genotype vs. RR+RX combined) was 3.76 (95%CI: 1.82-7.39; P=0.0002). Our findings indicated that the α-Actinin-3 gene rs1815739 RR genotype was more prevalent in the sprinters and distance athletes. In the bodybuilders, cyclists, and ironmen it was found that they were harboring the RR and RX genotype equally. According to the results, we suggest the α-Actinin-3 rs1815739 as a potential biomarker for personal training programs.

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Introduction

Athletic performance is the key to success during a sporting event. Environmental factors, which include regular training, nutrition, and combining the latest developments in technology are important for the physical performance of athletes. One of the most important factors affecting performance is genetic differences. The glucose/lipid metabolism, muscular strength, blood lactate threshold and maximal oxygen uptake (VO2max) are also some of the genetic factors that affect the capacity for different sporting disciplines (1). The studies about endurance performance also showed that increasing mitochondrial capacity and improving muscle glycogen levels are obtained by high-intensity training. The prediction of which athlete would be world-class could be achieved after all relationships between biological factors, recovery, and performance are elucidated (2).

α -actinin (ACTN) is a major component of the skeletal muscle. There are four different ACTN proteins in humans. *ACTN2* and *ACTN3* encode skeletal muscle α -actinins (3), The *ACTN3* gene encodes α-actinin-3, which is a sarcomeric actin-binding protein that is essential for generating powerful contractions (4) and is related to athletic performance. The common results of studies are that athletic performance is polygenic and the most susceptible relationship is provided by the *ACTN3* gene (5). A transition (C>T) at the nucleotide position 1747 in the *ACTN3* coding sequence converts an arginine (R) to a stop codon (X) at residue 577 (rs1815739) (6), and this change results in a truncated and non-functional protein in individuals which are carrying X allele (7). On the other hand, the presence of the R allele is accordingly thought to improve the

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quality and speed of the constriction and is fundamentally more typical in running competitors (8).

Although a lot of papers on the connection between *ACTN3* genes and accomplishments in sports have been published in recent years, this subject is still regarded as not clarified (9). The results of these studies are inconsistent. Despite the ethnicity, gender, training types and other factors that may affect the results, we tried to determine the genotyping differences in the players from diversified sports categories. Thus, we conducted a study on a group of athletes from different sporting areas. The study aimed to assess if a certain *ACTN3* genotype proves this cohort.

Materials and methods

Participants

A total of 131 athletes (39 Turkish cyclists, 34 sprinters and distance athletes, 33 volleyball players, 15 bodybuilders, and 10 ironmen), all with Turkish ancestry were enrolled in the study. All participants in the elite atlete group were national and international athletes. As a control group, 89 sedentary individuals were conditioned to not be connected with any particular type of sport, without a regular exercise program. All the athletes who had been actively exercising within the past 5 years were and all members of sports clubs. They were current and past non-smokers, 15 or more hours of training per week, with no concomitant diseases and also with no known genetic diseases. Body weight (BW) and body height (BH) were measured. The body mass index (BMI) was calculated. The "Informed Volunteer Consent Form Sample" was used to inform the athletes who participated in our research about the study's scope and substance. Consent forms were signed and written consent was obtained with the athletes' awareness.

Genetic analysis

The Genomic DNA from the venous blood was isolated by using the PureLink DNA isolation kit (Invitrogen, Van Allen Way Carlsbad, Calif., USA) by following the manufacturers' instructions. The genotyping of the *ACTN3* rs1815739 polymorphism was performed using a reverse transcription-quantitative PCR on a QuantStudio 3 (Thermo Fisher Scientific, Inc.) using a TaqMan Genotyping assay according to the manufacturer's protocol (cat. no. 4362691; Thermo Fisher Scientific, Inc.). Based on the genotypes, the subjects were divided into three groups: 1) *ACTN3* XX group (TT genotype), 2) *ACTN3* RR group (CC genotype), 3) *ACTN3* RX group (CT genotype).

Statistical analysis

The association of the polymorphisms with athletic status was evaluated by conducting the following contrasts: RR (reference group) vs. RX, and RR vs. XX (co-dominant effect); RR vs. RX and XX combined (dominant effect); RR and RX combined (reference group) vs. XX (recessive effect). The genotype distribution and allele frequencies between the groups of athletes and non-athletic controls were then compared by χ^2 testing using the GraphPad InStat statistical package. The p values of <0.05 were considered statistically significant.

Ethics committee

The Uskudar University Ethics Committee approved the study protocol (B.08.6.YÖK.2 .ÜS.0.05.0.06/2013/09). The procedures followed in the study were conducted ethically according to the principles of the World Medical Association Declaration of Helsinki and ethical standards in sport and exercise science research. All the participants signed the consent form and provided all the pertinent information.

Results and discussion

All of our athletes in different disciplines who participated in our study were successfully genotyped. Anthropometric characteristics of elite athletes and sedentary control group are shown in Table 1. No significant were discovered differences in age, height, weight and body mass index between the two groups.

Table 1. Anthropometric characteristics of the elite athletes and sedentary control group (mean ± SD)

	n	Age	Height (cm)	Weight (kg)	BMI (kg/m^{-2})
Sprinter runners	20	20.32±4.2	175.8±5.2	69.5±3.2	22.6±2.1
Distance runners	14	19.2 ± 1.8	173.5±3.8	61.6±5.3	20.3±1.7
Bodybuilders	15	22.2±3.6	182.5±6.8	87.6±2.1	25.6±3.4
Road cyclists	39	27.5±4.8	186±4	69±5.8	21.18±2.4
Ironmen	10	33 ± 3	180±1.2	77.4±8.6	23.5±1.9
Volleyball players	33	20.8±4.6	197.4±2.3	91	23.3±3.1
Controls	89	32.6±4.8	166.08±9.09	58.08±9.63	20.2±0.86

The genotype and allele number distributions of our athletes are summarized in Table 2.

Table 2. Genotype and allele distributions of ACTN3 R577X genotype in the professional athletes

Group	n	ACTN3 Genotypes			*p-value*	Alleles		*p-value*
		RR	RX	XX		R	X	
Sprinter and distance athletes	34	15 (44)	12 (35)	7 (21)	0.3143	42 (62)	26 (38)	0.46
Bodybuilders	15	7 (47)	7 (47)	1 (6)	0.2078	21 (70)	9 (30)	0.647
Cyclists	39	12 (31)	13 (33)	14 (36)	0.0006*	37 (47)	41 (53)	0.004*
Ironmen	10	4 (40)	3 (30)	3 (30)	0.0128*	11 (55)	9 (45)	0.081
Volleyball Players	33	5 (15)	21 (64)	7 (21)	0.0001*	31 (47)	35 (53)	0.004*
Totals	131	43 (33)	56 (43)	32 (24)	0.0500	142 (54)	120 (46)	0.06
Controls	89	42 (47)	36 (40)	11 (13)	1.00	120 (67)	58 (33)	1.0

Absolute and relative data are in the table. Data within parentheses are relative values. (*p<0.05 indicates the statistically significant difference.)

Table 3 displays the association of the *ACTN3* R577X polymorphism with athletic status. The odds ratio (OR) of cyclists harboring the XX vs. the RR genotype (co-dominant effect) when compared with the sedentary controls was 4.19 [95% confidence interval (CI): 1.87-9.18; p= 0.0002]. The OR of having the RR genotype vs. having the dominant trait (RX+XX combined) was 0.5 (95%CI: 0.28–0.91; p=0.02), while the OR of having the XX genotype vs. having the recessive trait (RR+RX combined) was 3.76 (95%CI: 1.82-7.39; p=0.0002). The results presented for the cyclists were statistically significant.

The OR for volleyball players in terms of the RX vs. the RR genotype (co-dominant effect) was 5.01 (95%CI: 2.45-10.2; p=0.0001), and the XX vs. RR genotype (co-dominant effect) was 5.062 (95%CI: 2.04-12.59; p=0.0003). In addition to this, having the RR genotype vs. having the dominant trait (RX+XX combined) was 0.19 (95%CI: 0.1-0.38; p=0.0001) for the volleyball players. All of the results were statistically significant.

When we compared the ironmen and the control group in terms of the XX versus the RR genotype (codominant effect), the OR value was 2.71 (2.71 (95%CI: 1.26-5.89; p=0.01), whereas the OR of the XX genotype vs. having the recessive trait (RR+RX combined) was 2.86 (95%CI: 1.42-5.65; p=0.003). When we compared all the groups with controls in terms of the XX vs. the RR genotype (co-dominant effect), the OR value was 2.69 (95%CI: 1.16-6.03; p=0.017). These results were statistically significant.

Table 3. The odds ratio of ACTN3 R577X genotypes in the professional athletes

	Sprinters and distance	Bodybuilders	Cyclists	Volleyballers	Ironmen	Totals
RR (ref.)	1.0	1.0	1.0	1.0	1.0	1.0
RX	0.93 (0.51;1.76)	1.17 (0.66; 2.1)	1.25 (0.65;2.42)	5.01 (2.45;10.2)	0.88 (0.46;1.64)	1.53 (0.8;2.81)
p	0.828	0.588	0.497	<0.0001*	0.695	0.176
XX	1.72 (0.76; 3.91)	0.46 (0.16;1.31)	4.19 (1.87;9.18)	5.062 (2.04;12.59)	2.71 (1.26;5.86)	2.69 (1.16;6.03)
p	0.181	0.142	0.0002*	0.0003*	0.01*	0.017*
RX+XX vs. (RR ref.)	0.88 (0.5; 1.55)	1.0 (0.56; 1.75)	0.5 (0.28; 0.91)	0.19 (0.1; 0.38)	0.75 (0.43; 1.32)	0.55 (0.31; 1)
p	0.67	>0.99	0.02*	<0.0001*	0.318	0.043
XX vs. RR+RX (ref.)	1.77 (0.86; 3.68)	0.42 (0.15; 1.12)	3.76 (1.82; 7.39)	1.77 (0.86; 3.68)	2.86 (1.42; 5.65)	2.11 (1; 4.28)
p	0.132	0.091	0.0002*	0.13	0.003*	0.045

(*p<0.05 indicates the statistically significant difference.)

The opinion that genetic makeup is closely linked with the capacity for human physical activity has been generally acknowledged in recent decades (10,11). Various examinations have affirmed the relationship between the *ACTN3* 577R allele and muscle power phenotypes (for example running time and fiber type), and physical capabilities in people; one duplicate of the *ACTN3* 577R allele is advantageous for cooperation in running and power sports (12), while there is a reliably potent relationship between the RR genotype and elite power performance (13). In addition to these studies, the main findings of Eynon et al. (14) research were that elite power athletes having the XX genotype is less likely and more prevalent in endurance athletes. In another study conducted with Spanish basketball players, the genotypes percentages of RR, RX, XX were found to be 37%, 42%, and 21%, respectively (15).

In the meta-analysis studies conducted on the genetic basis of athletic performance, it has been stated that the *ACTN3* 577RR genotype is associated with sprint / power-oriented performance tendency in the Caucasian race (16,17). On the contrary, some studies indicate that the R577X polymorphism is not predisposed to certain athletic performance (18). In addition, Norman et al. (19) suggested that there was no effect of the R577X polymorphism in *ACTN3* to differences in, fatigability, power output, or force-velocity characteristics in moderately trained athletes and that α -actinins do not play a significant role in determining muscle fiber-type composition. They suggested that α-actinin 2 might compensate for the deficiency of α -actinin-3. In another study, designed the study to find any association between *ACTN3* R577X genotype and injury epidemiology. They found no statistically significant value for XX genotype. Despite of elite, they reported that in their study, athletes carrying the RR genotype were more prone to injuries. Many studies have been carried out on genetic regions, which are thought to affect athletic performance and athletes belonging to different sports disciplines such as football, basketball, judo, and wrestling (21).

The studies involving *ACTN3* R577X analyses in terms of sports genetics in Turkey are less in number when compared to investigations from other countries. Günel et al. (2014) found the RX genotype as the highest genotype value as a percentage in both the athlete group and sedentary controls. They explained that the higher X allele in these individuals is the predisposition of this allele in sports requiring endurance (22). In a study involving elite athletes from the Aegean region, the *ACTN3* R577X analysis of 105 athletes from different disciplines such as track and field, baseball, judo, taekwondo, wrestling, cycling, football, and tennis was conducted. According to the analysis results, when compared with the RR and RX genotypes in the athlete group and sedentary individuals, it was stated that they were statistically significantly different (23).

Polat et al. (24) also found similar results to our investigation. They reported The *ACTN3* RX genotype and R allele were dominant in their study group of Turkish bodybuilders and stated that the *ACTN3* rs1815739 polymorphism could be an important determinant for a tendency of athletes in particular sports. Ulucan et al. (25) evaluated the *ACTN3* R577X polymorphism in trained Turkish middle-school children’s sprinting performance. They observed an RR genotype and R allele dominancy in the genetic distribution of trained children. In a different cohort study, In the study cohort, the *ACTN3* T allele, a value that is associated with the endurance phenotype, despite of there is no statistically significant, was found to be more dominant in professional football players (26). Eroglu et al. (27) tried to discover whether or not the ACTN*3* polymorphism is advantageous for sportive performance. They divided national and amateur Turkish runners into three groups according to distances, which were short, medium, and long. In contrast to our results, they detected no *ACTN3* RR polymorphism in national athletes. The RX genotype was more common in all the groups. On the other hand, in the amateur athletes and controls, they reported that the R allele was more common.

In conclusion, our findings provide support for an association between *ACTN3* R577X polymorphism and elite athletic status in a group of different sport types. The findings indicated that the *ACTN3* RR genotype is more prevalent in sprint and distance athletes, and bodybuilders, cyclists, and ironmen harbor the RX genotype equally. Comparing controls with the cyclists, ironmen, and volleyballers, the values we obtained were statistically significant. Studies have shown that the formation and development of athletic performance is multifactorial, which is determined by the interaction of environmental factors and different gene groups. It is very difficult to determine which genetic factor affects physical performance and also hard to identify whether environmental factors or genetic factors are more effective. However, studies with plentiful data are needed to determine these genotype effects and also genotype-environment interactions should be investigated. Future studies are promoted to enroll large enough samples of elite athletes from Turkey.

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Authors' contributions

Author MD and author KU have given substantial contributions to the conception or the design of the manuscript, author BTA to acquisition, analysis and interpretation of the data. Author BTA and author KU contributed for collection and analysis of data. MD and KU participated to drafting the manuscript, author KU revised it critically. All authors read and approved the final version of the manuscript. All authors contributed equally to the manuscript and read and approved the final version of the manuscript.

Interest conflict

The authors declare that they have no conflict of interest.

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