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Association between Sport Performance and Alpha-Actinin-3 Gene R577X Polymorphism

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ABSTRACT Physical performance has been associated with many gene variants including the alpha-actinin-3 gene (*ACTN3*) R577X polymorphism. The purpose of the present study is to investigate whether there is an association between sport performance of Turkish elite athletes and alpha-actinin-3 gene R577X polymorphism. A total of 300 individuals (150 elite athletes and 150 sedentary individuals) participated in the study voluntarily. A 291-bp long region spanning the R577X polymorphic site of the alpha-actinin-3 gene was amplified and tested with polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) method. The statistical analyses were done by using SPSS 15.0 package program. Although, no significant difference was found between the distributions of the three genotypes of elite athletes and sedentary individuals, the frequency of XX genotype in sedentary individuals was lower than that of the elite athletes ($p>0.05$). The RX and XX frequencies were significantly different ($P<0.05$) between the two groups. To the best of the researchers' knowledge, there are no reports that examined the ACTN3 polymorphism in the Athletes who live in Black sea region of Turkey

INTRODUCTION

Elite athletes are defined as the ones who have competed at a national or international level in a given sport (Macarthur and North 2005). The concept that genetic traits are strongly associated with human physical performance has been accepted by both scientific and sport communities in the past decade. For instance, it was suggested that the heritability of athlete status was estimated to be approximately 66% (De Moor et al. 2007). In the recent years, researchers have focused on how to find the exact genetic profiles that contribute to sport performance. They also conducted studies on how to determine the underlying mechanisms involved in specific fields of elite athletic performance in different branches. One of the main aims of such studies is to recognize individuals with genetic potential to be elite athletes and to help in giving trainers guiding information within this context (Yang et al. 2003). The determination of a potential performance in sport at an early age will set the ground that will help the athletes to choose the right sport. This will also enable the athletes to accomplish optimum success. To make this happen, performance criteria should be set for different branches and talent selections should be made accordingly (Tutkun et al. 2006).

As for 2009, more than 200 genetic variants have been associated with physical performance and the most studied one of these is alpha-actinin-3 (*ACTN3*) gene (Bray et al. 2009). This gene encodes for alpha actinin 3, a structural sarcomeric protein that can be found in fast type II muscle fibers which is used during explosive activities. R577X (rs1815739) polymorphism which results in the formation of a nonsense codon and hence a shorter polypeptide was first defined by North et al. (North et al. 1999). The *ACTN3* gene is located on chromosome 11q13-q14. A C>T change occurring at the nucleotide 1747 in exon 16 changes the arginine amino acid (R) to a stop codon (X). It has been suggested that the short form of the protein does not cause any disease and that more than one billion people might have this polymorphism (MacArthur and North 2007).

Since RR genotype is more frequently seen in elite power athletes (Yang et al. 2003), it is considered to be an advantage in power performances because it is also an R allele carrier. XX genotype is associated with low sprint ability and muscle strength (Yang et al. 2009). In 2003,

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Yang et al. showed that there is a significant association between ACTN3 genotype and athletic performance. When male and female elite sprinters were compared with controls, the frequencies of 577 R alleles in elite sprinters were significantly higher than those of the controls (Yang et al. 2003).

In a recently published meta-analysis which examined the relationship between *ACTN3* gene and sportive performance, the individuals who carried R allele were found to have the advantage in modalities that required explosion and muscle strength which supports the general consistency of the association between power based athletic performance, and ACTN3 genotype (Ma et al. 2013). Another meta-analysis supported the view that speed and power athletes have higher frequencies of RR genotypes (Alfred et al. 2011). In addition, studies conducted in Taiwan, Israel and Greece reported an association between RR genotype and elite power performance (Papadimitriou et al. 2008; Eynon et al. 2009; Chiu et al. 2011).

It has also been reported that ACTN3 XX genotype frequency varies significantly among different populations. Yang et al. reported that XX genotype was observed with a low frequency in Kenyan population (<1%) whereas, it was observed with a high frequency in Australian Caucasians (18%) and Japanese (24 %) (Yang et al. 2007). The purpose of this study is to test whether there is an association between alpha actinin 3 gene polymorphism and sportive performance in a population lives in the Black Sea Region.

MATERIAL AND METHODS

Study Population

The blood samples from elite athletes and sedentary individuals were collected with the approval of Ondokuz Mayis University Faculty of Medicine Scientific Researches Ethic Committee. The blood samples of elite athletes and sedentary individuals were taken at Ondokuz Mayis University Faculty of Medicine Central Laboratory. In this study, the group consisted of 150 elite athletes (18 females and 132 males) from different sport branches (football, basketball, athleticism, volleyball, handball, judo, wrestling, taekwondo, rugby), representing Ondokuz Mayis University Yasar Dogu Faculty of Sport Sciences. The control group (93 females and 57 males) consisted of voluntary sedentary individuals studying at Ondokuz Mayis University. For DNA isolation, 2 ml peripheric blood was taken from each individual of the research and control group in tubes that contained EDTA. DNA isolation was carried out using DNA isolation kit (Vivantis GF-1, Malaysia) as recommended by the manufacturer.

Determination of Genotypes

ACTN3 gene polymorphism was studied by PCR–RFLP method. In PCR, 5'-CTG TTG CCT GTG GTA AGT GGG -3' was used as forward primer and 5'-TGG TCA CAG TAT GCA GGA GGG -3' was used as reverse primer. PCR was carried out in a reaction mixture of 25 µl which contained 100ng genomic DNA, 200µM of each dNTP, 2 units of Taq DNA polymerase and 10 pmol from each primer. PCR protocol used include: initial denaturation at 94°C for 5 minutes; 30 seconds at 94°C, 1 minute at 60°C, 1 minute at 72°C, 35 cycles and last extension at 72°C for 7 minutes.

The amplified product of PCR (291 bp long) was cut with Dde I restriction enzyme (RE). For endonucleolytic cut, a 28 µl reaction mixture that contained 15 µl bidistilled water, 10 µl PCR product, 2 µl 10 X fastdigest Green Buffer and 1 µl fastdigest *Dde* I was prepared. After the mixture was left for incubation at 37°C for one hour in a water bath, it was run in a 3.5% agarose gel and the DNA bands were visualized using the gel imaging system, thereafter, the results were analyzed. In RR individuals, as a result of RE cutting the PCR product once, bands of 86 and 205 base pairs (bp) were expected; and in XX individuals, as a result of RE cutting the PCR product twice because of the R577X mutation, bands of 108, 97 and 86 bps were expected. RX individuals show all of the four DNA bands: 205, 108, 97 and 86 bps.

Statistical Analysis

The statistical analyses were done by using SPSS 15.0 package program. The frequencies of the alleles and genotypes in patients and controls were compared with χ^2 analysis. Odds ratio (OR) and 95% confidence intervals (CIs) were calculated. p values less than 0.05 (two-tailed) were regarded as statistically significant. Power

analysis was carried out by using Minitab 15.0 package program.

RESULTS

With regard to the allele frequencies (n=300), p= 36.6%, and p0= 47.4%, Post-Hoc power analysis evaluated the power of the study as 76.6%.

In this study, the average age, height and weight, respectively, were 21.8 years, 177.3cm and 75.2kg for elite athletes and 20.3 years, 168.4cm and 62.5kg for sedentary individuals (Table 1). ACTN3 R577X genotype and allele frequencies of elite athletes and sedentary individuals in Black Sea Region are summarized in Table 2. XX genotype frequency was found to be the higher in elite athletes than sedentary individuals; however, this difference was not statistically significant. No significant difference was found between the genotype frequencies of elite athletes and sedentary individuals ($p>0.05$).

Table 1: Age, height and weight distribution of sedentary individuals and elite athletes

Group	*n*	*Average*	*Standard deviation*	*t-test*
Age (Years)				
Sedentary	150	20.3	1.30	-7.1 **
Elite athletes	150	21.8	2.30	
Height (cm)				
Sedentary	150	168.4	9.00	-8.5**
Elite athletes	150	177.3	8.80	
Weight (kg)				
Sedentary	150	62.5	11.90	-8.7**
Elite athletes	150	75.2	13.10	

**P < .001

ACTN3 577 R allele frequency was found to be significantly different between elite athletes and sedentary individuals ($p<0.05$). In sedentary individuals, the frequency of R allele was found to be higher than X allele (63.3%; 36.6%).

This study revealed a statistically significant difference between the RR and RX genotype distributions of elite athletes and the sedentary individuals ($p<0.05$). In sedentary individuals, XX genotype was found to be significantly lower than RR genotype (Table 3).

It can be seen from Table 4 that when the RR+RX and XX genotype frequencies of elite athletes and sedentary individuals were examined, a statistically significant difference was found ($p<0.05$). XX genotype was found to have a higher frequency in elite athletes (28.22%) than in sedentary individuals (12.67%). Conversely, RR+RX genotype was found to have a higher frequency in sedentary individuals.

DISCUSSION

Athletic performance is known to be associated with training, environmental factors and genetic predisposition. *ACTN3* is a member of actin binding protein family and it presumably affects sport performance. In general population, no significant difference has been reported between R577X allele frequency of men and women. However, a gender related association has been reported between ACTN3 genotype and athletic or muscular performance (Papiarni et al. 2007). This study tested the role of ACTN3 R577X polymorphism on the performance of elite athletes in a population of those living in the Black Sea Region.

Table 2: Genotype and allele frequencies of elite and sedentary individuals

Genotype/ Allele	*Elite athletes (n=150)*		*Sedentary individuals (n=150)*		*÷²*	*p value*	*OR (95 %CI)*
	n	*%*	*n*	*%*			
Genotype							
RR	46	30.6	59	39.3	÷²=5.37	p=0.068	
RX	71	47.3	72	48.0			
XX	33	22.0	19	12.6			
Total	150		150				
Allele	*n*	*%*	*n*	*%*			
R	163	52.5	190	63.3	÷²=6.80[a]	p=0.009*	0.64 (0.46-0.90)
X	147	47.4	110	36.6			
Total	300		300				

Yates corrected
* P <0.05

Table 3: RR-XX genotype distributions of elite athletes and sedentary individuals

Genotype/ Allele	*Elite athletes (n=150)*		*Sedentary individuals (n=150)*		*÷²*	*p value*	*OR (95 %CI)*
	n	*%*	*n*	*%*			
Genotype							
RR	46	58.2	59	75.6	÷²=4.62[a]	p=0.03*	0.45 (0.21-0.94)
XX	33	41.8	19	24.4			
Total	79		78				

[a] Yates corrected
* P <0.05

Table 4: RR+RX and XX genotype distributions of elite athletes and sedentary individuals

Genotype/ Allele	*Elite athletes (n=150)*		*Sedentary individuals (n=150)*		*÷²*	*p value*	*OR (95 %CI)*
	n	*%*	*n*	*%*			
Genotype							
RR+RX	117	71.78	131	87.33	÷²=10.56[a]	p=0.0011*	0.37 (0.20-0.69)
XX	46	28.22	19	12.67			
Total	163		150				

[a] Yates corrected
* P <0.05

When the genotype distributions were reviewed, RR was 30.6%, RX was 47.3% and XX was 22.0% for elite athletes while RR was 39.3%, RX was 48.0 % and XX was 12.6% for sedentary individuals. Although, there was no significant difference between the RR-RX-XX genotype distributions of elite athletes and sedentary individuals, XX genotype was found to have a lower frequency in sedentary individuals when compared with elite athletes; however, this difference was not statistically significant (p>0.05).

Allele frequency of elite athletes was 52.5% for R and 47.4 % for X while the allele frequency of sedentary individuals was 63.3% for R and 36.6 % for X. According to this data, the difference between the allele frequencies of elite athletes and sedentary individuals was found to be statistically significant (p<0.05). R allele was found to be significantly higher for sedentary individuals while X allele was found to be significantly higher for elite athletes (47.4%; 36.6%). Similarly, XX genotype was found to have the higher frequency in elite athletes when compared with sedentary individuals. When RR+RX genotype and XX genotype were compared, it was found that RR+RX genotype was significantly higher in sedentary individuals while XX genotype was significantly higher in elite athletes (p = 0.0011).

Similar results were noted in a study involving 155 Israeli athletes (endurance runners and sprinters) and 240 sedentary individuals. In this study which compared national top sprinters, it was reported that top sprinters were found to have a higher frequency of R allele while endurance athletes were found to have a higher frequency of XX genotype (34%) when compared with controls (18%; p=0.02) and sprinters (13%; p=0.002). The same study asserted that ACTN3 R allele was associated with high level sprint performance and X allele and XX genotype were associated with endurance performance (Eynon et al. 2009).

Eynon et al. (2014) also studied the association between the α-actinin-3 (*ACTN3)* R577X polymorphism and elite team-sport athletic status in three cohorts of European team-sport athletes (205 team-sport athletes, 305 endurance athletes, 378 sprint/power athletes). The researchers reported that the ACTN3 R577X polymorphism was not associated with team-sport athletic status, compared to endurance athletes and non-athletic controls (n = 568).

Yang et al. (2003) in their study with 107 white Australian endurance athletes found highly significant associations between ACTN3 genotypes and athletic performance.

Mikami et al. (2014) studied the association between the α-actinin-3 (*ACTN3)* R577X poly-

morphism and athletic performance in 299 elite Japanese track and field athletes (134 sprint/ power athletes; 165 endurance/ middle-power athletes) and 649 Japanese controls. They reported that ACTN3 R577X genotype was associated with sprint/power performance in elite Japanese track and field athletes, especially with short sprint performance.

In a study which examined the ACTN3 genotype and allele frequency distributions of Taiwanese elite speed swimmers, it was found that R allele and RR+RX genotype frequency had a higher frequency in general population; however, this difference was not statistically significant. Nevertheless, when international and national performances were analyzed, it was found that R allele frequency was significantly higher in female international sprint swimmers (67.6%) when compared with national sprint swimmers (50.0%) and general population (53.7%). On national level, R allele in general population was found to be higher than that in national sprint swimmers (Chiu et al. 2011). In our study, when elite athletes and sedentary individuals were analyzed in terms of RR+RX genotype frequency, sedentary individuals were found to have statistically higher frequency of RR+RX genotype than elite athletes (Table 4).

A study with Lithuanian elite athletes found no significant difference between control group and athletes in terms of genotype and allele frequencies; however, when ACTN3 XX genotype was compared between the control group and athletes, similar to the findings of this study, athletes were found to have higher frequencies. The researchers stated that functional *ACTN3* deficiencies in fast muscle fibrils can be regulated mostly by ACTN2 in anaerobic muscle metabolism or other enzymes (Ginevièienë et al. 2011).

Garatachea et al. (2014) examined the association of the ACTN3 R577X polymorphism with leg-muscle explosive power in Spanish elite basketball players (n=100) and non-athletic controls (n= 283). Although, the ACTN3 R577X polymorphism was associated with explosive muscle performance (this phenotype is important for a basketball player, especially, during jumps), yet they found no association with leg explosive power in elite basketball players or with the status of being this type of athlete.

In a study by Papadimitriou et al. (2008), 181 controls and 101 Greek elite athletes were examined in terms of ACTN3 R577X genotype and power athletes were found to have statistically different ACTN3 genotype and allele frequencies when compared with the control group. RR genotype was found to have a higher frequency in athletes when compared with the general population.

In a study involving 125 voluntary healthy North Indian blood donors, the frequencies of ACTN3 R and X alleles were determined. Of the 125 donors, 22% were RR, 61% RX, and 17% were XX (Goel and Mittal 2007). The results indicated that, the *ACTN3* gene RR, RX and XX genotype frequencies, as well as the North Indian and the white populations were similar (Goel and Mittal 2007).

In another study involving 450 controls and 250 endurance athletes, the *ACTN3* gene XX genotype frequency in endurance athletes was found to be very high compared with those of the other groups (Shang et al. 2010).

Tural et al. (2014) studied PPAR-α and PPARGC1A gene variants in 60 Turkish elite level endurance athletes and 110 sedentary controls and found statistically significant differences between genotypic and allelic frequencies of the athletic and control groups. Ulucan and Göle (2014) also from Turkey, investigated ACE I/D polymorphism in 8 Turkish elite wind surfers and concluded that this polymorphism might serve as a biomarker in identifying the predisposition to certain kind of sports.

A study conducted in Aegean Region found that the RR, RX and XX genotype frequencies were 25.7%; 34.3% and 40.0%, respectively for sedentary individuals and 32.4%; 53.3% and 14.3%, respectively for elite athletes. RR and RX genotype distributions of elite athletes were found to be significantly different from those of sedentary individuals ($p<0.01$) (Sanlisoy et al. 2011). Sanlisoy et al. found that the sedentary individuals had an R allele frequency of 42.4% and an X allele frequency of 57.6% while elite athletes had an R allele frequency of 60.5% and an X allele frequency of 39.5% and that the R and X allele distribution of elite athletes was significantly different from those of sedentary individuals ($p<0.01$) (Sanlisoy et al. 2011). The R and X allele frequency of elite athletes results of our study were similar with the results of Sanlisoy et al.'s (2011) study, while the allele frequency results of sedentary individuals were

different from the results of their study. The difference in these results seems to suggest that there may be gene prevalence differences between the populations of Aegean and Black Sea regions.

CONCLUSION

The present study indicated that the genotype and allele frequencies of the elite athletes were significantly different from those of the sedentary individuals. The researchers are of the opinion that this study which was the first in Black Sea Region will lead to other studies on determining the distribution of ACTN3 alleles and the selection of athletes in Turkey. The researchers also hope that this molecular study will positively contribute to the development of sports, in general.

RECOMMENDATIONS

Studying the association of individual gene of interest with physical performance seems to limit our scope. Thus, the researchers recommend that whole-genome association studies should be undertaken in order to cover the whole genome and hopefully identify additional candidate genes of importance.

LIMITATIONS

There are mainly two limitations in our study. First, the researchers have only analyzed the most common polymorphism in the ACTN3 gene, because of financial constraints the researchers were unable to assay other polymorphisms. Finally, the size of the researchers' cohort was not large enough to improve the analysis power of this study due to the insufficient number of available elite athletes. The researchers hope that future studies will address these limitations.

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