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ACTN3 R577X genotype and performance of elite middle-long distance swimmers in China

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ABSTRACT: The *ACTN3* gene is one of the genes that have a potential influence on physical performance. Studies have shown that the *577R* genotype of *ACTN3* is more prevalent in sprint athletes, while the *577X* genotype is more prevalent in endurance athletes. In swimming, both power and endurance related phenotypes are equally needed for swimmers to excel at the elite level. Therefore, the *ACTN3 R577X* polymorphism may become a genetic marker for swimmers. The study aimed to examine the association of the *ACTN3 R577X* genotype with the performance of elite middle-long distance (MLD) swimmers. The distributions of the *ACTN3 R577X* (*rs1815739*) genotype and allele were examined in a general population (206) and a group of elite MLD swimmers (160) in China by using PCR-RFLP and TOF. Compared with the general population, the elite MLD swimmers, especially the females, had a higher frequency of the *RR* genotype. The swimmers had a higher frequency of the *R* allele than the general population. However, the difference was not statistically significant. After being stratified by performance, the difference of the *R* allele frequency between the international master athletes and the general population was statistically significant. The elite MLD swimmers had a higher frequency of the *RR* genotype than the *RX+XX* genotype compared with the general population. The *ACTN3 R577X* polymorphism was associated with the performance of elite MLD swimmers in China. The SNP *R577X* could be used as a biomarker for selecting elite MLD swimmers in China.

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INTRODUCTION

In the past 20 years, the study of genetics and elite athletic performance has made considerable progress, with many genetic markers having been found [1]. Most studies so far have focused on predominantly endurance or power athletes, who represent the physiological end-points of the sporting continuum. However, the genetic contribution to success in sports that require a combination of aerobic and anaerobic qualities (e.g., middle-long distance swimming) has received limited attention [2].

The *ACTN3* gene encodes the protein α-actinin-3, which is almost exclusively expressed in the sarcomeres of the fast glycolytic type II fibres generating rapid forceful contractions during activities such as sprinting and weightlifting. Results from the ACTN3-knockout (KO) mouse model suggest that α-actinin-3 may affect muscle mass and muscle glycogen levels [3]. Indeed, the KO mice had a higher level of activity in the aerobic oxidative pathway and a lower level of activity in the anaerobic glycolytic pathway compared with their wild-type counterparts. They also had lower muscle mass, stronger fatigue resistance, larger diameter of fast-twitch (IIB) muscle fibres and weaker muscular strength [4].

A polymorphism in the *ACTN3* gene (*R577X*) results in a lack of α-actinin-3 in the *XX* genotype. The *R* allele is a normal version of the gene, whereas the *X* allele contains a sequence change that stops the production of the functional α-actinin-3 protein [5]. Although the mechanistic link has not been established, the percentage area and the number of type IIx fibres were greater in the *RR* than in the *XX* genotype, and the level of α-actinin-3 protein was systematically higher in type IIx fibres than in type IIa fibres [6]. The nonsense mutation of the alpha-actinin-3 gene was not associated with dystrophinopathy [7], so this deficiency did not result in a disease phenotype or muscular functional impairment. On the other hand, many studies have indicated that the *ACTN3 R577X* genotype was associated with power-oriented (e.g., sprinters, jumpers, throwers) athletic performance among Caucasians. The prevalence of the mutated *X* allele was lower among power-oriented athletes compared with nonathletic referents or endurance athletes, indicating that the lack of α-actinin-3 was detrimental in these sports [8-9]. In contrast, the association between the *ACTN3 R577X* polymorphism and the performance of elite endurance athletes was not conclusive [8]. While

some studies indicated that endurance athletes presented a higher frequency of the *XX* genotype compared with the general population [10], other studies did not show any significant difference in genotype frequencies between endurance athletes and nonathletic referents [11-12].

Studies showed that the *ACTN3 R577X* polymorphism is associated with power and endurance related phenotypes. Swimming is an activity where both power and endurance related phenotypes are equally needed for swimmers to excel at the elite level. Therefore the *ACTN3 R577X* polymorphism may become a genetic marker for swimming. However, the study of the *ACTN3 R577X* polymorphism and MLD swimmers has received limited attention. This study examined the association of the *ACTN3 R577X* genotype with the performance of elite MLD swimmers by comparing a group of elite MLD swimmers with a general population of the same ethnic origin in China. The hypothesis was that the *ACTN3 R577X* genotype is associated with the elite MLD swimmers' performance.

MATERIALS AND METHODS

Ethical approval

This study was approved by the Ethics Review Board for Human Studies at Beijing Sport University. All the participants signed an informed consent document. The study conformed to the principles of the Declaration of Helsinki.

Subjects

The study included (i) a general population of 206 healthy nonathletes with no self-reported family history of competitive sports participation and (ii) 160 elite MLD swimmers (≥ 400 m, ≤ 1,500 m). All the subjects were recruited from an area north of Huaihe River (altitude ≤ 800 m) and are of Han origin of at least 3 generations.

The general population (118 men, 88 women) were undergraduates aged 20±1 years from China Agricultural University. The elite MLD swimmers, awarded the title "National Master Athlete" by the General Administration of Sport of China, were the best Chinese athletes in 800 m freestyle swimming (81 women) and 1500 m freestyle swimming (79 men). They were 20±2 years old (men) and 19±1 years old (women). All the female swimmers clocked less than 9 min in 800 m freestyle and 17 min 25 s in 1,500 m freestyle. The male swimmers clocked less than 8 min 30 s in 800 m freestyle and 16 min 10 s in 1,500 m freestyle. 18 men and 20 women reached the level of "International Master Athlete" defined by the General Administration of Sport of China: best performance ≤ 15 min 19.22 s (men) and 16 min 48.81 s (women) in 1500 m freestyle; ≤ 8 min 6.55 s (men) and 8 min 36.32 s (women) in 800 m freestyle.

5 mL of venous blood was collected into EDTA-K2 tubes (BD). Within 24 h of collection, the blood was centrifuged and separated into plasma, buffy coat cells, and red blood cells before being stored in a -80°C freezer.

Genotyping

The conventional method was used for extracting DNA from the peripheral venous blood. The *ACTN3* genotypes of the general population and swimmers were determined using the PCR-RFLP technique and time of flight mass spectrometry respectively [13-14]. The gene sequencing method was used to judge the accuracy of the test results. The test results are shown in Fig. 1.

Statistical analysis

SPSS 13.0 for Windows was used for the data analysis. The distribution of the *ACTN3* genotypes of the general population was tested to determine if it was in Hardy-Weinberg equilibrium by using a chi-square test with 1 degree of freedom. The distributions of the *ACTN3* alleles and genotypes of the elite swimmers and the general population were compared using chi-square tests. The level of significance was set at 0.05.

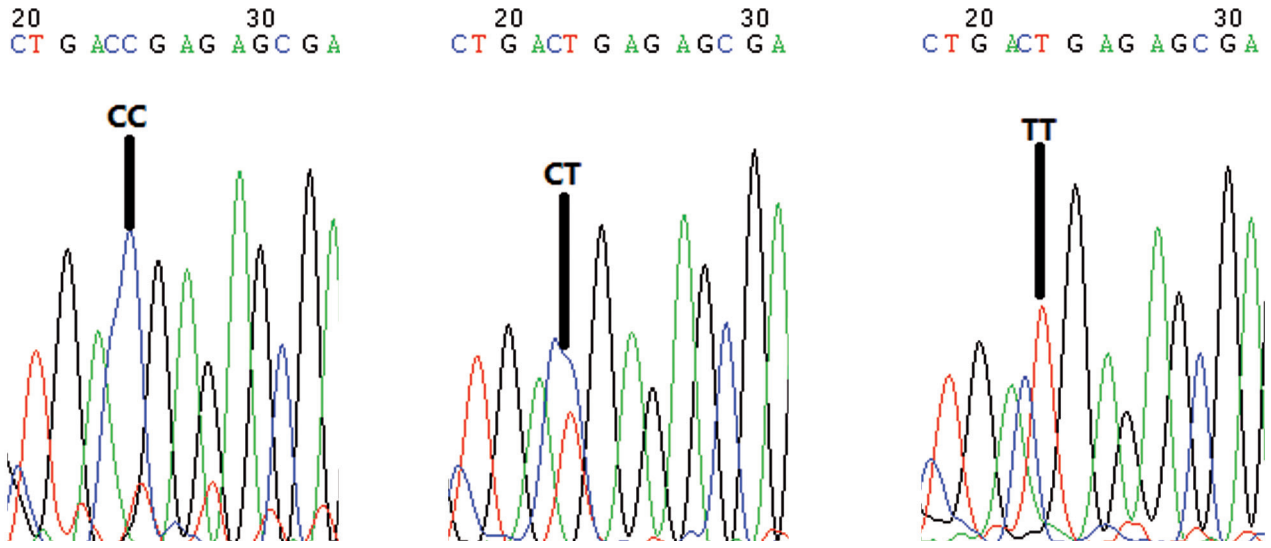

FIG. 1. Three possible *ACTN3* genotypes at position 577 by gene sequencing.

TABLE 1. Frequencies of *ACTN3* genotypes among the general population and the athletes.

	RR	RX	XX	RR+RX	RX+XX	R	X
	N(%)	N(%)	N(%)	N(%)	N(%)	N(%)	N(%)
The general population							
Total (206)	64(31.1)	104(50.5)	38(18.4)	168(81.6)	142(68.9)	232(56.3)	180(43.7)
Male (118)	36(30.5)	58(49.2)	24(20.3)	94(80.7)	82(69.5)	130(55.1)	106(44.9)
Female (88)	28(31.8)	46(52.3)	14(15.9)	74(84.1)	60(68.2)	102(58.0)	74(42.0)
Athlete							
Total (160)	71(44.4) *	58(36.2)	31(19.4)	129(80.6)	89(55.6)^	200(62.5)+	120(37.5)
Male (79)	33(41.8)	30(38)	16(20.2)	63(79.7)	46(58.2)	96(60.8)	62(39.2)
Female (81)	38(46.9)	28(34.6)	15(18.5)	66(81.5)	43(53.1)	104(64.2)	58(35.8)
IM Athlete							
Total (38)	20(52.6)**	14(36.9)	4(10.5)	34(89.5)	18(47.4)^^	54(71.1)++	22(28.9)
Male (18)	9(50)	8(44.4)	1(5.6)	17(94.4)	9(50)	26(72.2)	10(27.8)
Female (20)	11(55)	6(30)	3(15)	17(85)	9(45)	28(70)	12(30)

Note: Values are absolute (relative frequencies in parentheses), Athlete: National and International Mater Athlete, IM Athlete: International Master Athlete

$^{*}\chi^2(2)$ = 8.487, $p < 0.05$, genotype frequency, Athletes vs. The general population

$^{\wedge}\chi^2(1)$ = 6.850, $p < 0.05$, RR and RX+XX genotype frequency, Athletes vs. The general population

$^{+}\chi^2(1)$ = 2.853, $p < 0.1$, R and X Allele frequency, Athletes vs. The general population

$^{**}\chi^2(2)$ = 6.737, $p < 0.05$, genotype frequency, IM Athletes vs. The general population

$^{\wedge\wedge}\chi^2(1)$ = 6.608, $p < 0.05$, RR and RX+XX genotype frequency, IM Athletes vs. The general population

$^{++}\chi^2(1)$ = 5.748, $p < 0.05$, R and X Allele frequency, IM Athletes vs. The general population

RESULTS

The distributions of the *ACTN3* alleles and genotypes of the swimmers and the general population are summarized in Table 1. The *ACTN3 R577X* genotype frequencies met Hardy- Weinberg expectations in the general population. When the elite MLD swimmers were compared with the general population in a single test, a significant association was found for *ACTN3*. No association was found for *ACTN3* by gender.

The frequency of the *ACTN3 577R* allele was higher among the elite MLD swimmers than in the general population. However, the difference was not statistically significant. When stratified by performance, the difference was statistically significant. No statistically significant difference was found when stratified by gender. The frequency of the *ACTN3 RR + RX* genotype had no significant difference between the elite MLD swimmers and the general population. The frequency of the *RR* genotype was significantly higher than that of the *RX+XX* genotype in the elite MLD swimmers than in the general population.

DISCUSSION

The study hypothesized that the *ACTN3* polymorphism affects the performance of elite MLD swimmers. In fact, this study observed associations between the *ACTN3 R577X* polymorphism and the elite MLD swimmers' performance. The frequencies of the *ACTN3 R577X* genotypes were different between the general population and the swimmers, suggesting that this polymorphism can significantly influence the performance of the elite MLD swimmers. The results showed that the *ACTN3 RR* genotype was more frequent among the elite MLD swimmers, especially among the female swimmers, than in the general population. However, previous studies showed different results [13,15,16,17]. The *ACTN3 R577X* genotype was not a genetic marker for identifying talented Taiwanese swimmers [13], because the limited performance level and the swimmers (≤ 400 m swimming competitions) were different from this study (because short-distance swimmers (400 m) with all performance levels were chosen), while the best MLD swimmers were selected in this study. Other studies [15-17] were also inconsistent with our results. Different ethnicities may be another reason causing this difference. Although the *ACTN3 R577X* genotype was associated with Israeli swimmers [10], its frequencies were significantly different from that found in this study. These discrepancies reflect the possibility that there may be an interaction between genotypes and ethnic backgrounds. Thus, future genetic studies should focus more on ethnic backgrounds.

RR was the superior genotype of the elite MLD swimmers in this study, its frequency being higher than that of other genotypes. Swimmers with the *RR* genotype would more likely become an elite swimmer. Previous studies showed that the *RR* genotype was dominant in power-oriented athletes [8-9]. It was also found to be significant in sports requiring both aerobic and anaerobic qualities (e.g., long-distance swimming). Fibre type characteristics were significantly different between the *RR* and the *XX* genotype groups. The percent-

age surface area and number of type IIx fibres were greater in the *RR* genotype group than in the *XX* genotype group, and the α-actinin-3 protein content was systematically higher in type IIx fibres than in type IIa fibres [18]. Athletes with the *RR* genotype had high levels of testosterone [19]. This may explain, in part, the association between the *ACTN3 RR* genotype, skeletal muscle hypertrophy and power athlete status, so athletes with the *RR* genotype were more likely to be elite athletes, especially in sports requiring both aerobic and anaerobic qualities.

Most studies found that the *ACTN3 577R* allele was more frequent in power-oriented athletes [8-9]. The *ACTN3 R* allele enhanced high velocity muscle tasks of healthy young men [18], while the mean testosterone levels were significantly higher in both males and females with the *ACTN3 R* allele than in the *XX* homozygotes [19]. Athletes with the *577R* allele were more likely to become elite athletes [20]. Although this study did not find that the *R* allele is a dominant allele among the swimmers, it was higher than in the general population. However, the association did not reach statistical significance. When stratified by performance, the differences between international master athletes and the general population were statistically significant. Consistent with this finding, the *R* allele was significantly higher in the Taiwanese female international sprint swimmers compared with the national level sprint swimmers and the general population [14]. In these studies further analysis was not performed, because the performance data of the samples were imprecise [15-17]. Further studies are required to determine how the *R* allele affected swimming performance. In the near future, the *R* allele may be the best biomarker in selecting talented MLD swimmers.

Where both power and endurance-related phenotypes are needed to excel at the elite athletic level, the energy supply system of MLD swimmers mainly combines aerobic metabolism and the glycolysis system, which requires speed, strength and aerobic endurance [16]. Considering the potential benefits of the *R* allele for power/sprint athletic performance, and of the *X* allele for endurance athletic performance, it would be biologically logical that the *RX* genotype may play a key role in swimming performance where both power and endurance phenotypes are important [10]. However, there are few studies examining the potential heterozygote effect of the *ACTN3* genotype. The results of this study were not consistent with those studies [13,15,16,17]. Although the frequency of the *RR* genotype was significantly higher among the elite athletes than in the general population, the distribution was not significantly different between the *RR*+*RX* genotype and the *XX* genotype. However, the study found that the distribution of the *RR* and *RX*+*XX* genotype was significantly different between the elite MLD swimmers and the general population, which was inconsistent with the results of most other studies [21]. The main reason for this difference was the distribution frequency of the *RX* genotype. The frequency of the *RX* genotype in the athletes was lower, particularly in the elite female athletes, which is similar to the finding of Ruiz [16]. Future studies are needed to confirm these findings. Previous studies indicated that the *RR* genotype influences speed and muscle strength [8-9], while the *XX* genotype dominantly influences muscle endurance [10-11]. Research on the association of the *RX* genotype with athletic performance is limited. Future research should, however, focus on investigating whether the *RX* genotype confers any advantage in muscle power over the *XX* genotype, or in muscle endurance over the *RR* genotype.

This study revealed that the frequency of the *ACTN3 RR* genotype was higher among the elite female swimmers compared with the general population, and the frequency of the *RR* genotype among the female swimmers was higher than that of other genotypes, which was especially true among those who were International Master Athletes. No differences were observed in men. The frequency of the *ACTN3 R* allele was also significantly higher among the elite Taiwanese female swimmers compared with the general population, yet no differences were observed in men [13]. Yang for the first time reported that none of the Olympians or female power athletes had the *XX* genotype [16]. Taiwanese late adolescent girls with the *ACTN3 RR* genotype performed significantly better in terms of handgrip strength than those with other genotype combinations [22]. Women with the *ACTN3 XX* genotype have weaker lower body strength than those with the *RX* genotype [23-24]. These studies all supported this finding. However, the genotype frequencies of this study were not different between genders. The mechanism of the high *RR* frequency in females was not clear. Therefore, this study cannot explain the high *RR* frequency in women. Maybe there is an unknown linkage to another functional variation close to *ACTN3*, which requires further study.

CONCLUSIONS

The *ACTN3 R577X* polymorphism was significantly associated with the performance of the elite MLD swimmers. The *RR* genotype was higher among athletes. The SNP *ACTN3 R577X* polymorphism could be one of the biomarkers for identifying talented MLD swimmers in China.

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