Eur J Appl Physiol (2008) 103:631–634
DOI 10.1007/s00421-008-0763-1

ORIGINAL ARTICLE

Association of the *ACTN3* R577X polymorphism with power athlete status in Russians

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Accepted: 28 April 2008 / Published online: 10 May 2008

Abstract The α-actinin-3 (*ACTN3*) gene encodes a Z-disc structural protein which is found only in fast glycolytic muscle fibers. A common nonsense polymorphism in codon 577 of the *ACTN3* gene (R577X) results in α-actinin-3 deficiency in XX homozygotes. Previous reports have shown a lower proportion of the *ACTN3* XX genotype in power-oriented athletes compared to the general population. In the present study we tested whether XX genotype was underrepresented in Russian power-oriented athletes. The study involved 486 Russian power-oriented athletes of regional or national competitive standard. *ACTN3* genotype and allele frequencies were compared to 1,197 controls. The frequencies of the *ACTN3* XX genotype (6.4 vs. 14.2%; $P < 0.0001$) and X allele (33.3 vs. 38.7%; $P = 0.004$) were significantly lower in power-oriented athletes compared to controls. Furthermore, the lowest (3.4%) frequency of the *ACTN3* XX genotype was found in a group of highly elite athletes, supporting the hypothesis that the presence of α-actinin-3 has a beneficial effect on the function of skeletal muscle in generating forceful contractions at high velocity. In conclusion, *ACTN3* R577X polymorphism was associated with power athlete status in Russians.

Keywords α-actinin-3 · Genotype · Fast-twitch fibers · Power performance

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Introduction

Although the human genome has now been sequenced, the influence of gene polymorphisms on genetic predisposition to sports is largely unknown. Numerous studies were conducted concerning the determination of association of the α-actinin-3 gene (*ACTN3*) polymorphism with human physical performance and elite athlete status. The searching for such kind of connection is based on the function of α-actinins in skeletal muscle fibers. They constitute the predominant protein component of the sarcomeric Z line, where they form a lattice structure that anchors together actin containing thin filaments and stabilizes the muscle contractile apparatus (Squire 1997). Moreover, interacting with many muscle proteins α-actinins carry out some signaling and metabolic functions. Expression of the α-actininins-3 is limited to fast muscle fibers responsible for generating force at high velocity (Mills et al. 2001; Vincent et al. 2007).

C-to-T transition in exon 16 of the *ACTN3* gene leads to a stop-codon (R577X polymorphism), which results in no *ACTN3* protein detectable in muscle fibers (North et al. 1999). But the complete deficiency of the α-actinin-3 in 577X homozygotes does not result in a disease phenotype (North et al. 1999; Suminaga et al. 2000). The first research of the R577X polymorphism in athletes demonstrated that the frequency of the 577X null allele is significantly lower in elite sprint and power athletes than in controls, suggesting that α-actinin-3 is required for power performance (Yang et al. 2003). Several reports had confirmed this association (Niemi and Majamaa 2005; Papadimitriou et al. 2007; Roth et al. 2008; Santiago et al. 2008) and were supported by a number of cross-sectional studies which could provide some data to indicate that there is a positive association between the presence of the R-allele and the capacity to perform high power muscle contractions (Clarkson et al.

2005; Delmonico et al. 2007; Moran et al. 2007; Vincent et al. 2007). Furthermore, recently Vincent et al. (2007) have shown that the percentage surface and number of type IIx (fast-twitch glycolytic) fibers was greater in the RR than the XX genotype group of young healthy men.

The aim of the present study was to examine the association between *ACTN3* R577X polymorphism and power athlete status in Russians.

Materials and methods

The University of St Petersburg Ethics Committee approved the study and written informed consent was obtained from each participant.

Subjects and controls

Four hundred and eighty-six male and female Russian athletes of regional or national competitive standard were recruited from the following sports: alpine skiing ($n = 29$), artistic gymnastics ($n = 44$), bodybuilding ($n = 23$), figure skating ($n = 10$), ice hockey ($n = 34$), jumping events ($n = 8$), powerlifting ($n = 9$), running 100–400 m ($n = 70$), ski jumping ($n = 18$), soccer ($n = 4$), speed skating ($n = 90$), swimming 50–100 m ($n = 10$), throwing events ($n = 15$), volleyball ($n = 9$), weightlifting ($n = 55$) and wrestling ($n = 58$). Sport-specific strength development required for these sports/events is shown in Table 1. Twenty-nine athletes were classified as "highly elite", being at least winners of the World Championships, World Cups and Olympic Games; 71 athletes were classified as "elite", being at least silver or bronze medalist of the World Championships, World Cups and Olympic Games or prize winners of Europe Championships; 206 athletes were classified as "sub-elite" (participants of international competitions), the others ($n = 180$) were classified as "average" athletes, being regional competitors with no less than 4 years experience participating in their sports.

Controls consisted of 1,197 healthy unrelated citizens of St Petersburg, Moscow, Naberezhniye Chelny and Surgut (524 males and 673 females). The athletes and control groups were all Caucasians. Further characteristics are presented in Table 2.

Genotyping

DNA was extracted from mouthwash samples as previously described (Bolla et al. 1995). Genotyping for the C1743T (R577X) variant was performed by polymerase chain reaction (PCR) and restriction enzyme digestion. PCR primers were forward CTGTTGCCTGTGGTAAGTGGG and reverse TGGTCACAGTATGCAGGAGGG, generating a fragment of 290 bp. PCR products were digested with *BstDEI* (SibEnzyme, Russia) for 12 hours at 60°C and were separated by 8% polyacrylamide gel electrophoresis, stained with ethidium bromide, and visualized in UV light.

Statistical analysis

Allele frequencies were determined by gene counting. Genotype distribution and allele frequencies between

Table 1 Sport-specific strength development required for sports/events

Sport/event	Types of strength required
Alpine skiing	Reactive power, muscular endurance (M-E) of short duration
Artistic gymnastics	Reactive power, takeoff power, landing power
Bodybuilding	Absolute power, M-E of short duration
Figure skating	Takeoff power, landing power, power-endurance
Ice hockey	Acceleration power, deceleration power, power-endurance
Jumping events	Acceleration power, takeoff power, reactive power
Powerlifting	Absolute power, reactive power
Running, 100–400 m	Reactive power, starting power, acceleration power, power-endurance
Ski jumping	Takeoff power, reactive power
Soccer	Reactive power, acceleration/deceleration power, M-E of short/medium duration
Speed skating	Starting power, acceleration power, M-E of short/medium duration, power-endurance
Swimming, 50–100 m	Starting power, acceleration power, M-E of short duration
Throwing events	Throwing power, reactive power
Volleyball	Reactive power, power-endurance, throwing power
Weightlifting	Reactive power, absolute power
Wrestling	Power-endurance, throwing power, M-E of medium duration

Table 2 *ACTN3* genotype distribution of the athletes and controls with sex (frequencies) and age

	ACTN3 genotype			*P* value	X allele (%)	*P* value
	RR (%)	RX (%)	XX (%)			
Athletes						
All, $n = 486$	39.7	53.9	6.4*	<0.0001*	33.3	0.004*
Male, $n = 363$	37.7	55.9	6.4*	<0.0001*	34.3	0.021*
Female, $n = 123$	45.5	48.0	6.5	0.067	30.5	0.034*
Age, years	24 ± 0.7	24.5 ± 0.6	24.1 ± 1.0			
Controls						
All, $n = 1197$	36.8	49.0	14.2	–	38.7	–
Male, $n = 524$	36.8	46.8	16.4	–	39.8	–
Female, $n = 673$	36.8	50.7	12.5	–	37.8	–
Age, years	17.1 ± 0.2	17.2 ± 0.2	16.7 ± 0.4			

* $P < 0.05$, statistically significant differences. Comparison with controls was by χ^2 test

RR Wild-type homozygote, *RX* heterozygote, *XX* mutant homozygote

groups of athletes and controls were then compared by χ^2 test using GraphPad InStat statistical package. *P* values of <0.05 were considered statistically significant.

Results

ACTN3 genotype distribution amongst controls was in Hardy–Weinberg equilibrium ($\chi^2 = 0.6$; $df = 2$, $P = 0.74$). Genotype distribution amongst controls (RR 36.8%, RX 49.0%, XX 14.2%) was similar to that observed in several reported groups of Caucasian populations (Moran et al. 2007; North et al. 1999; Yang et al. 2003). No difference was found in genotype and allele frequencies within groups of controls from diverse cities of Russia (data not shown).

ACTN3 genotype distribution and X allele frequency amongst athletes are presented in Table 2. Hardy–Weinberg equilibrium calculation showed deviation from expected frequencies in athletes ($\chi^2 = 11.5$; $df = 2$, $P = 0.003$). Genotype distribution in a whole cohort of athletes showed significant difference ($P < 0.0001$) compared to controls. The frequencies of the *ACTN3* XX genotype (6.4 vs. 14.2%; $P < 0.0001$) and X allele (33.3 vs. 38.7%; $P = 0.004$) were significantly lower in athletes compared to controls.

ACTN3 XX genotype frequency significantly correlated with elite athlete status (Fig. 1). We found a decreasing linear trend of XX genotype with increasing athletes' level ($P < 0.0001$ for linear trend). There was only one athlete (world record holder in hammer throwing) with XX genotype amongst highly elite athletes ($n = 29$).

We also investigated the association of the *ACTN3* R577X polymorphism with athlete status in male and female athletes. *ACTN3* X allele frequencies in both men (34.3 vs. 39.8%, $P = 0.021$) and women (30.5 vs. 37.8%, $P = 0.034$) were significantly different compared to controls. Furthermore, XX genotype was under-represented in both sexes (males: 6.4 vs. 16.4%, $P < 0.0001$; females: 6.5 vs. 12.5%, $P = 0.067$) compared to controls (Table 2).

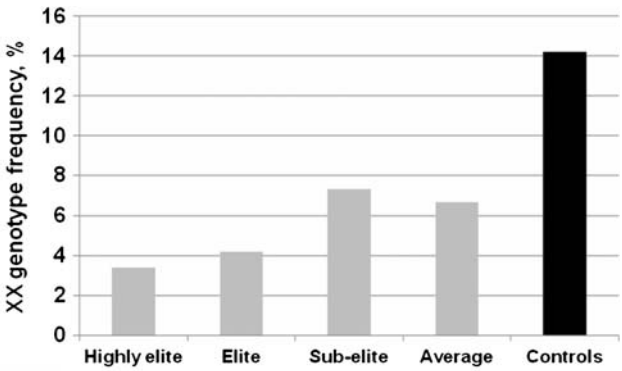

Fig. 1 *ACTN3* XX genotype frequency amongst power-oriented athletes with different level and sedentary controls is shown. XX genotype frequency in controls was 14.2%. By comparison, it was 3.4, 4.2, 7.3 and 6.7% for highly elite, elite, sub-elite and average athletes, respectively ($P < 0.0001$ for linear trend)

Discussion

The frequencies of *ACTN3* genotypes and alleles in Russian population have not been previously examined. Here we show that the distribution of *ACTN3* genotypes and alleles in Russians is similar to that observed in several reported groups of Caucasian populations (Moran et al. 2007; North et al. 1999; Yang et al. 2003).

Our data suggest that the *ACTN3* RR and RX genotypes are associated with predisposition to power sports and positively correlated with elite power athlete status in Russians. It seems that the *ACTN3* R allele provides an advantage for strength and sprint performance because the *ACTN3* XX genotype is significantly reduced in elite and highly elite power-oriented athletes compared to controls. The finding of significant deviations from Hardy–Weinberg equilibrium in the athletes but not in controls in our study is consistent with a true genotype association (Wittke-Thompson et al. 2005), as it was also reported by Roth et al. (2008) in considering strength athletes.

The results of the present investigation are in agreement with previously reported case–control studies which provide evidence that *ACTN3* RR genotype is over-represented or *ACTN3* XX genotype is under-represented in strength/sprint athletes in comparison with controls. More specifically, Yang et al. (2003) for the first time have shown that the frequency of the *ACTN3* XX genotype was reduced in Australian power athletes (6 vs. 20%) compared to controls, whereas none of the Olympians or female power athletes had an XX genotype. These findings have been supported by the independent replications in case–control studies of elite Finnish sprint athletes (frequency of XX genotype: 0 vs. 9.2%) (Niemi and Majamaa 2005), elite Greek track and field athletes (frequency of RR genotype: 47.94 vs. 25.97%) (Papadimitriou et al. 2007), top-level professional soccer players, participating in the Spanish Championships (frequency of RR genotype: 48.3 vs. 28.5%), elite-level strength athletes from across the United States (frequency of XX genotype: 6.7 vs. 16.3%) (Roth et al. 2008).

Although these results indicate that the presence of α-actinin-3 in fast-twitch fibers has a beneficial effect on success in sprint/strength events, it seems that the carriage of RR or RX genotype is not an absolute criterion for being an elite power athlete. At least two reports (including present study) show that α-actinin-3 deficiency is compatible with elite power athlete status. Lucia et al. (2007) have reported the case of a Spanish elite long jumper (two times Olympian) whose genotype for the *ACTN3* gene is XX. We have also observed one highly elite Russian hammer thrower (world record holder) with such genotype.

The possible mechanisms underlying association of the *ACTN3* R577X polymorphism with power performance have been discussed in detail elsewhere, and include recent findings that the percentage surface and number of type IIx fibers were greater in the RR than the XX genotype group (Vincent et al. 2007), and that muscle from α-actinin-3 knockout mice displays reduced force generation (MacArthur et al. 2008).

In summary, we have shown that variation in the *ACTN3* gene is strongly associated with elite power athlete status in Russians. Such findings have important implications for our understanding of molecular mechanisms underlying the predisposition to high power potential, and support the hypothesis that the presence of α-actinin-3 has a beneficial effect on the function of skeletal muscle in generating forceful contractions at high velocity (Yang et al. 2003).

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