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Paper

The *ACTN3* R577X polymorphism in Russian endurance athletes

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Abstract

Objective: The functional 577R allele of the α-actinin-3 (*ACTN3*) gene has been reported to be associated with elite power athlete status, while the nonfunctional 577XX genotype (predicts an α-actinin-3 deficient phenotype) has been hypothesized as providing some sort of advantage for endurance athletes. In the present study we examined the distribution of *ACTN3* genotypes and alleles in Russian endurance-oriented athletes and looked for association between *ACTN3* genotypes and the competition results of rowers.

Methods: The study involved 456 Russian endurance-oriented athletes of regional or national competitive standard. *ACTN3* genotype and allele frequencies were compared to 1,211 controls. The data from the Russian Cup Rowing Tournament were used to search for possible association between the *ACTN3* genotype and the long-distance (~ 6 km) rowing results of 54 athletes. DNA was extracted from mouthwash samples. Genotyping for the R577X variant was performed by PCR and restriction enzyme digestion.

Results: The frequencies of the *ACTN3* 577XX genotype (5.7% vs. 14.5%; $P < 0.0001$) and 577X allele (33.2% vs. 39.0%; $P = 0.0025$) were significantly lower in endurance-oriented athletes compared to the controls, and none of the highly elite athletes had the 577XX genotype. Furthermore, male rowers with *ACTN3* 577RR genotype showed better results (1339 ± 11 s) in long-distance rowing than carriers of 577RX (1386 ± 12 s) or 577XX (1402 ± 10 s) genotypes (P=0.016).

Conclusion: Our data show that the *ACTN3* 577X allele is underrepresented in Russian endurance athletes and is associated with the rowers' competition results.

Keywords: alpha-actinin-3, genotype, endurance performance

Introduction

It has long been recognized that the interindividual variability of physical performance traits has a strong genetic basis. The polymorphisms in genes that influence these traits are now being sought. One gene potentially associated with human physical performance is the *ACTN3* gene, which encodes the protein α-actinin-3. This protein forms part of the sarcomeric apparatus in the fast glycolytic fibres of human skeletal muscle – the fibres responsible for the generation of rapid, forceful contractions in activities such as sprinting and weightlifting and is thought to perform specialized roles important to the functioning of these fibres (for review, see[1]).

A common genetic variation in the *ACTN3* gene (C-to-T transition in exon 16; rs1815739) that results in the replacement of an arginine (R) with a stop codon (X) at amino acid 577 (R577X) had been identified nearly a decade ago.[2] This variation creates two different versions of the *ACTN3* gene, both of which are common in the general population: the 577R allele is the normal, functional version of the gene, whereas the 577X allele contains a sequence change that completely prevents the production of functional α-actinin-3 protein. More than a billion people worldwide have two copies of the nonfunctional 577X variant (the XX genotype), resulting in the complete deficiency of α-actinin-3 protein in their skeletal muscle.[1]

Several studies demonstrated that the frequency of the 577XX genotype is lower in elite sprint and power athletes than in controls, suggesting that α-actinin-3 is required for a power performance.[3-8]

Recently two studies reported that the loss of α-actinin-3 expression in a knockout mouse model results in a shift in muscle metabolism toward the more efficient aerobic pathway and an increase in intrinsic endurance performance.[9,10] However, little is known about the effect of α-actinin-3 deficiency on human endurance performance. The hypothesis of Yang et al.[3] that α-actinin-3 deficiency may confer some advantage in endurance performance events, based on the case-control study of Australian endurance athletes, has not been supported by the independent studies of elite endurance athletes from different countries.[4,11-14]

The aim of the present study was to examine the distribution of *ACTN3* R577X genotypes and alleles in Russian endurance athletes and to search for possible associations between the *ACTN3* genotype and the rowers' competition results.

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 fifty six male and female Russian athletes of regional or national competitive standard were recruited from the following endurance sports: biathlon (n = 40; distances: 15-20 km), cross-country skiing (n = 98; distances: 10-50 km), race walking (n = 21; distances: 10-50 km), road cycling (n = 34; distances: ≥50 km), rowing (n = 187, distances: ≥2000 m), swimming 0.8-25 km (n = 42) and triathlon (n = 34; distances: swimming 1500 m, cycling 40 km, running 10 km). Thirty athletes were classified as 'highly elite', being at least winners of the World Championships, World Cups and Olympic Games; ninety nine athletes were classified as 'elite', being at least silver or bronze medalists at the World Championships, World Cups and Olympic Games or prize winners at European Championships; one hundred and five athletes were classified as 'sub-elite' (participants of international competitions), the others (n = 222) were classified as 'average' athletes, being regional competitors with no less than 4 years experience participating in their sports.

The data (rowing time, sec) from the Russian Cup Rowing Tournament (October 14-15, 2006; rowing canal "Don", Rostov-on-Don) were used to search for possible associations between the *ACTN3* genotype and the long-distance rowing performance of 54 highly elite and elite athletes (34 males (19 rowers from double scull teams and 15 rowers from single scull teams) and 20 females (12 rowers from double scull teams and 8 rowers from single scull teams). The Russian Cup Rowing Tournament is a non-standard long-distance (predominantly aerobic) race competition which covers a

distance of approximately six km. The official results of the Russian Cup Rowing Tournament 2006 are available at the following website: www.rowingru.com.

Controls were 1,211 healthy unrelated citizens of St Petersburg, Moscow, Naberezhniye Chelny and Surgut (532 males and 679 females). The athletes and control groups were all Caucasians (predominantly Russians and Tatars), with an equivalent ratio of European and Siberian descent (3:1 in both groups) for more than three generations. Further characteristics are presented in Table 1.

Genotyping

DNA was extracted from mouthwash samples as previously described.[15] 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

Genotype distribution and allele frequencies between groups of athletes and controls were compared by χ^2 testing using the GraphPad InStat statistical package. Differences between genotype groups for the investigated competition results were assessed using one-way ANOVA. *P* values of < 0.05 were considered statistically significant.

Results

Case-control study

ACTN3 genotype distribution in the control group was in Hardy-Weinberg equilibrium (χ^2 = 0.59; df = 2, *P* = 0.743). Genotype distribution amongst controls (577RR – 36.5%, 577RX- 49.0%, 577XX – 14.5%) was similar to that observed in several reported groups of Caucasian populations (Table 1).[2,3,16]

Table 1. *ACTN3* genotype distribution of the athletes and controls with sex (frequencies) and age

	ACTN3 genotype			577X allele,
	577RR, %	577RX, %	577XX, %	%
Athletes				
All, *n* = 456	39.3	55.0	5.7*	33.2*
Male, *n* = 293	40.3	52.9	6.8*	33.3*
Female, *n* = 163	37.4	58.9	3.7*	33.1
Age, years	24.3 ± 0.4	24.4 ± 0.3	24.2 ± 1.4	
Controls				
All, *n* = 1211	36.5	49.0	14.5	39.0
Male, *n* = 532	36.3	47.0	16.7	40.2
Female, *n* = 679	36.7	50.6	12.7	38.0
Age, years	17.1 ± 0.2	17.2 ± 0.2	16.7 ± 0.4	

Values are means ± SE

*P<0.01, statistically significant differences. Comparison with controls was by χ^2 test

577RR wild-type homozygote; 577RX heterozygote; 577XX mutant homozygote

The *ACTN3* genotype distribution and the 577X allele frequency amongst the athletes are presented in the table 2. Hardy-Weinberg equilibrium calculation showed deviation from the expected frequencies in athletes (χ^2=14.2; df=2, *P*=0.0008). Genotype distribution in a whole cohort of athletes

showed significant differences ($P < 0.0001$) when compared to controls. The frequencies of the *ACTN3* 577XX genotype (5.7% vs. 14.5%; $P < 0.0001$) and 577X allele (33.2% vs. 39.0%; $P = 0.0025$) were significantly lower in athletes compared to controls.

Table 2. *ACTN3* genotype distribution and frequencies of *ACTN3* gene 577X allele in Russian endurance athletes

Sport	*n*	*ACTN3 genotype, %*			*P value*	577X allele, %	*P value*
		577RR	577RX	577XX			
Biathlon	40	42.5	55.0	2.5*	0.1	30.0	0.13
Cross-country skiing	98	45.9	49.0	5.1*	0.019*	29.6	0.0093*
Race walking	21	33.3	52.4	14.3	0.95	40.5	0.97
Road cycling	34	47.1	52.9	0*	0.049*	26.5	0.049*
Rowing	187	32.1	62.6	5.3*	0.0002*	36.6	0.42
Swimming 0.8-25 km	42	52.4	30.9	16.7	0.059	32.1	0.21
Triathlon	34	35.3	64.7	0*	0.038*	32.3	0.33
Totals	456	39.3	55.0	5.7*	<0.0001*	33.2	0.0025*
Controls	1211	36.5	49.0	14.5	1.00	39.0	1.00

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

In considering individual sporting disciplines, biathletes ($P = 0.034$), cross-country skiers ($P = 0.0097$), road cyclists ($P = 0.01$), rowers ($P = 0.0003$) and triathletes ($P = 0.01$) had a significantly lower percentage of the *ACTN3* 577XX genotype compared to controls (14.5%). The frequency of 577X allele was significantly lower only in cross-country skiers and road cyclists compared to controls (Table 2).

None of the highly elite athletes had the *ACTN3* 577XX genotype ($P = 0.016$, compared to controls). Furthermore, the frequencies of the 577XX genotype were also lower in elite (9.1%), sub-elite (7.6%; $P = 0.056$, compared to controls) and average athletes (4.1%; $P < 0.0001$, compared to controls).

We also investigated the association of the *ACTN3* R577X polymorphism with athletic status in male and female athletes. *ACTN3* genotype distribution in both men ($P = 0.0003$) and women ($P = 0.003$) was significantly different compared to male and female controls, respectively. Furthermore, the 577XX genotype was under-represented in both sexes (males: 6.8% vs. 16.7%, $P < 0.0001$; females: 3.7% vs. 12.7%, $P = 0.0004$) compared to controls (Table 1).

Genotype-phenotype association study

Amongst the 54 rowers who participated in the Russian Cup Rowing Tournament, we found only three athletes with the *ACTN3* 577XX genotype. These athletes were involved in the men's double scull race (n=19) and showed the slowest rowing times (1402 ± 10 s; $P = 0.016$) when compared to athletes with 577RX (1386 ± 12 s) and 577RR (1339 ± 11 s) genotypes. No statistically significant differences were found in the competition results of carriers of the 577RR and 577RX genotypes in other rowing groups.

Discussion

This is the first study to demonstrate that the *ACTN3* XX genotype is significantly under-represented in Russian endurance-oriented athletes compared to controls and that it limits the potential of athletes to achieve successful results in endurance competitions. The finding of significant deviations from the Hardy-Weinberg equilibrium in the athletes but not in controls in our study is consistent with a true genotype association.[17] Although previous studies indicated that the presence of α-actinin-3 in fast-twitch fibres has a beneficial effect on success in sprint/strength events, it seems

that the α-actinin-3 deficiency may also negatively influence the power component of sports performance in endurance athletes.

It is well known that the physiological demands of modern endurance events are very high and endurance-oriented athletes are also required to perform very forceful muscle contractions during the competitively critical phases of the races despite the long duration of these events.[18] For instance, despite the long duration of daily stages (~ 5 hours on average), professional cycling has evolved into a power-oriented sport over the past few years, at least as far as the most critical phases of the competitions are concerned.[11] Furthermore, the very high speeds and near-maximal intensities at which endurance events are currently performed by top-level runners and skiers probably requires the ability to recruit type II fibres, i.e., expressing α-actinin-3 protein.[11] With regard to rowing, it is well established that rowers should exhibit excellent isokinetic strength and power. They utilise a unique physiological pattern of race pacing; they begin exertion with a vigorous sprint which places excessive demands on anaerobic metabolism followed by a severely high aerobic steady-state and a fast finish.[19] Data also indicate that the rowing times become approximately 0.7 sec faster per year.[20] Additionally, in most endurance events in which the races begin with a mass start, the strategies to win include covering the distance with the top participants of the race for as long as possible, and turning the long-distance fight into an exhausted sprint for the finish.

Therefore, we assume that although α-actinin-3 deficiency is associated with the preponderance of type I (slow-twitch) fibres in untrained human subjects[21] and with increased activity of multiple enzymes in the aerobic metabolic pathway of knockout mice,[10] such a condition may be considered as a limiting factor in the manifestation of power and strength in endurance events. At least our findings that none of the highly elite endurance-oriented athletes had the *ACTN3* 577XX genotype and the fact that male rowers with the α-actinin-3 deficiency phenotype showed the slowest rowing time (577RR homozygotes were faster than athletes with 577XX genotype by ~ 1 min) support this hypothesis. Despite the possible favourable effect of an α-actinin-3 deficiency on aerobic metabolism, we suppose that Russian endurance athletes with α-actinin-3 in their working muscles can attain a top-level endurance performance not only due to the advantage of generating forceful contractions at high velocity, but also because of the compensatory and additive effect of other genetic variants associated with endurance-related traits.

The possible mechanisms underlying the association of the *ACTN3* R577X polymorphism with athletic performance have been discussed in detail in the recent publications.[1,9,10]

The first published case-control study showed that the frequency of the *ACTN3* 577XX genotype was higher in Australian endurance athletes (n = 194) compared to controls, although this was only significant in females (n = 72).[3] However, the hypothesis that the α-actinin-3 deficiency may confer some advantage in endurance performance events has not been supported by the independent studies of elite Finnish (n = 40), Spanish (n = 102), Ethiopian (n = 76), Kenyan (n = 284) and Italian (n = 42) endurance athletes and Caucasian triathletes (n = 457).[4,11-14] Furthermore, no significant relationships were detected between *ACTN3* R577X genotypes and endurance-related traits such as VO_{2max} in Spanish or Russian endurance athletes.[11,22] On the contrary, recently Gómez-Gallego et al[23] have reported that professional road cyclists with the *ACTN3* 577RR/RX genotypes had significantly higher peak power output and ventilatory threshold (both are considered as endurance phenotypic traits) values than their XX counterparts. The results of the present investigation are not in agreement with the study of Australian female endurance athletes; that may be explained, in part, by using different sample sizes and by differences in the effect of *ACTN3* R577X genotypes on the athletic performance of different ethnic groups. Additional studies are needed to clarify this question.

Our study does have limitations. The number of rowers participating in the Russian Cup Rowing Tournament with available DNA was small. As in all such studies, extension to, and replication within other racial groups is proposed. Further, it is worth mentioning that performance in the rowing competition is unlikely to be reducible to a single phenotype trait: motivation, mental toughness, tactical astuteness, team coherence, status of maturity, decision making and other non-physiological factors do also determine success. Therefore, our data showing that *ACTN3* R577X

polymorphism was associated with the results of the rowing competition should be interpreted with caution.

In conclusion, our data suggest that α-actinin-3 deficiency may negatively influence sports performance in Russian endurance athletes.

Competing interests: none declared.

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