Available online at www.sciencedirect.com

SciVerse ScienceDirect

Journal of Exercise Science & Fitness 10 (2012) 12–15

www.elsevier.com/locate/jesf

Original article

ACTN3 R577X polymorphism in top-level Polish rowers

Pawel Cieszczyk [a,*], Marek Sawczuk [b], Agnieszka Maciejewska-Karlowska [b], Krzysztof Ficek [c]

[a] *Department of Physical Culture and Health Promotion, University of Szczecin, Poland*
[b] *Department of Genetics, University of Szczecin, Poland*
[c] *Galen Medical Center, Poland*

Received 24 February 2011; revised 26 May 2011; accepted 9 November 2011
Available online 5 June 2012

Abstract

The first evidence that a mononucleotide difference in DNA sequence was associated with power ability referred to the R577X polymorphism of the *ACTN3* gene, where translation (C > T) at nucleotide position 1747 in the *ACTN3* coding sequence converts an arginine (R) to a stop codon (X) at residue 577. In the present study, DNA polymorphism derived from the *ACTN3* gene was studied in Polish rowers to examine the hypothesis that the *ACTN3* genotype is associated with athletic performance. The study involved 80 male Polish rowers of a nationally competitive standard and 204 unrelated volunteers for controls. Genotype distribution among the whole group of athletes (53.8% RR, 38.8% RX, 7.4 XX% DD) was significantly different to that among controls (36.3% RR, 46.1% RX, 17.6%; $p = 0.01$). When only elite rowers were considered, p value for genotype distribution (56.8% RR, 37.8% RX, 5.4% XX) was 0.03. The genotype distribution among nonelite rowers (52.2% RR, 39.1% RX, 8.7% XX) was not significantly different to that among controls ($p = 0.09$). A significant excess of the 577R allele was noted in the whole cohort of rowers (73.11%, $p = 0.002$). This trend was similar when comparing with the controls (59.3%) the allele frequency in elite rowers (75.7%, $p = 0.007$) and nonelite rowers (71.7%, $p = 0.026$). In conclusion, our results are contrary to the hypothesis that the *ACTN3* 577X allele may have some beneficial effect on endurance performance in rowing. On the contrary, the *ACTN3* RX or RR genotypes seem to be a requirement for being an elite rower, therefore, identification of *ACTN3* polymorphism as a genetic marker for rowing talent should be interpreted with great caution.

Keywords: α-Actinin-3; Athletic performance; Genotype; Rowing

Introduction

The response of the human body to physical training is highly variable among individuals, and many studies have indicated that this response may be strongly influenced by genetic variation.[1] The first evidence that a mononucleotide difference in DNA sequence was associated with power ability referred to the R577X polymorphism of the *ACTN3* gene,[2] where the translation (C > T) at nucleotide position 1747 in the *ACTN3* coding sequence converts an arginine (R) to a stop codon (X) at residue 577.[3] 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.[4] The complete deficiency of the α-actinin-3 in 577X homozygotes does not result in a disease phenotype,[4,5] proven by the fact that > 1 billion people worldwide have XX genotype.[6]

The α-actinins are a family of actin-binding proteins related to dystrophin.[2,7] In skeletal muscle, α-actinin proteins are an important structural component of the Z disc, where they anchor actin thin filaments, helping to maintain the myofibrillar array.[8,9] Moreover, α-actinins, interacting with many muscle proteins, carry out some signaling and metabolic

* Corresponding author. Department of Physical Culture and Health Promotion, University of Szczecin, Al. Piastow 40B, Blok 6, 71-065 Szczecin, Poland. Tel.: +48505769630; fax: +48 91 444 2734.
E-mail address: cieszczyk@poczta.onet.pl (P. Cieszczyk).

doi:10.1016/j.jesf.2012.04.003

functions.[7] Mills et al[3] have reported that α-actinin-3 is the predominant fast fiber isoform in humans/human muscle. Furthermore, it is suggested that α-actinin-3 may promote the formation of fast-twitch fibers[10] or alter glucose metabolism in response to training.[2] Additionally, α-actinin-3 may be evolutionarily optimized for the minimization of damage caused by eccentric muscle contraction.[2]

Two sarcomeric α-actinin isoforms exist in humans: α-actinin-2 (which is expressed in all types of muscle fibers) and α-actinin-3 (which is expressed almost exclusively in fast, glycolytic type II fibers).[3,7] Although it is likely that α-actinin-3 protein has many similar roles to α-actinin-2,[3,7] some authors suggest that the *ACTN3* gene has been maintained in the genome because of functions independent of *ACTN2*.[2]

The 577R allele and 577RR genotype of the *ACTN3* gene are associated with top-level, power-orientated athletic performance in a wide variety of ethnic groups.[2,11–16] Additionally, there is a positive association between the presence of the R allele and the capacity to perform high-power muscle contractions.[17,18] Furthermore, Vincent et al.[10] have shown that the percentage surface and number of type IIx (fast-twitch glycolytic) fibers was greater in the RR than the XX genotype of young healthy men.

However, the role of the 577X allele and 577XX genotype in athletic performance has not yet been sufficiently clarified.[19] Some studies have reported that the loss of α-actinin-3 expression in a knockout mouse model results in a shift in fast muscle metabolism toward the more efficient aerobic pathway and an increase in intrinsic endurance performance.[20,21] It may be proof for the Yang et al[2] hypothesis, that the total deficiency of the α-actinin-3 protein may confer some beneficial effect on endurance performance. This hypothesis seems to be supported by the fact that the XX *ACTN3* genotype occurs at higher frequency in some groups of elite endurance athletes.[2,11,19] In contrast to the studies mentioned above, there has been recent evidence that the XX *ACTN3* genotype is not associated with endurance performance.[2,23–25]

The aims of the present study were to perform preliminary analysis of the possible importance of the *ACTN3* gene polymorphisms in elite Polish rowers and sedentary individuals, and represent the possible relationships between genotype and physical performance.

Materials and methods

Ethics committee

The Pomeranian Medical University Ethics Committee approved the study and written informed consent was obtained from each participant.

Participants and controls

Eighty male Polish rowers of a nationally competitive standard were recruited for this study. Thirty-seven of them were elite rowers (aged 28 ± 4.1 years); national representatives with no less than 10 years experience participating in the sport. Eleven of them were European Championship medalists, seven were World Championship medalists, and 14 were Olympic medalists. The second group of recruited athletes ($n = 46$) were nonelite rowers consisting of Polish men (aged 23 ± 3.1 years) who had competed regionally. To avoid selection bias, various methods were used to obtain the samples: targeting national rowing teams, and information provided by national coaching staff and athletes attending training camps.

The controls were 204 volunteers (healthy, sedentary, unrelated, male students of University of Szczecin, aged 22 ± 2.6 years). The athletes and controls were all Caucasian to reduce the likelihood of racial gene skew and to overcome any potential problems of population stratification.

Genotyping

The buccal cells donated by the participants were collected in Resuspension Solution (Sigma, Germany) with use of Sterile Foam Tipped Applicators (Puritan, USA). DNA was extracted from the buccal cells using GenElute Mammalian Genomic DNA Miniprep Kit (Sigma) according to the manufacturer's protocol. The 290-bp fragment of exon 15 of the *ACTN3* gene was amplified by polymerase chain reaction (PCR) using the forward primer CTGTTGCCTGTGGTAAGTGGG and the reverse primer TGGTCACA GTATGCAGGAGGG. The PCR reaction mix contained 1 μL (30–50 ng) DNA, 0.2 μL primers (10 pmol/μL), and 0.1 μL Taq Polymerase (5 U/μL). PCR was performed for 35 cycles (30 seconds each of denaturation at 94 °C, annealing at 68 °C, and extension at 72 °C). The amplified PCR fragments were subsequently digested with *Dde*I endonuclease (Fermentas, Lithuania), and the alleles 577R and 577X were distinguished by the presence (577X) or absence (577R) of a *Dde*I restriction site. Digestion of PCR products of the 577X allele yielded bands of 108 bp, 97 bp and 86 bp, whereas digestion of PCR products of the 577R allele yielded bands of 205 bp and 86 bp. Digested products were then electrophoresed on three agarose gels stained with ethidium bromide.

Statistical analysis

Genotype distribution and allele frequencies between groups of athletes and controls were compared and significance was assessed by χ^2 test using STATISTICA 8 statistical package. P values < 0.05 were considered statistically significant.

Results

ACTN3 genotype distribution among participants and controls was in Hardy–Weinberg equilibrium, making selection bias less likely. Genotype distribution of the control group (36.3% RR, 46.1% RX, 17.6% XX) was similar to that reported in previous studies of Caucasian populations.[2,4,15,26] The distribution of the *ACTN3* genotypes and alleles is given in Table 1.

Table 1
Genotype and allele frequencies of *ACTN3* R577X. These data are presented as absolute and relative values (within parentheses). The *p* values correspond to comparisons in genotype and allele frequencies between athlete groups and controls.

Group	n	*ACTN3* genotype			*p*	*ACTN3* allele		*p*
		RR	RX	XX		R	X	
All rowers	80	43	31	6	0.01	117	43	0.002
		53.8%	38.8%	7.4%		73.11%	26.9%	
Elite rowers	37	21	14	2	0.03	56	18	0.007
		56.8%	37.8	5.4%		75.7%	24.3%	
Nonelite rowers	46	24	18	4	0.09	66	26	0.026
		52.2%	39.1%	8.7%		71.7%	28.3%	
Controls	204	74	94	36	—	242	166	—
		36.3%	46.1%	17.6%		59.3%	40.7%	

The genotype distribution among the whole group of athletes (53.8% RR, 38.8% RX, 7.4 XX% DD) was significantly different to that among controls (36.3% RR, 46.1% RX, 17.6%; $p = 0.01$). When only elite rowers were considered, the *p* value for genotype distribution (56.8% RR, 37.8% RX, 5.4% XX) was 0.03. The genotype distribution among the nonelite rowers (52.2% RR, 39.1% RX, 8.7% XX) was not significantly different to that among the controls ($p = 0.09$).

A significant excess of the 577R allele was noted in the whole cohort of rowers (73.11%, $p = 0.002$). This trend was similar when comparing with the controls (59.3%) the allele frequency in elite (75.7%, $p = 0.007$) and nonelite (71.7%, $p = 0.026$) rowers.

The genotype distribution among the elite and nonelite rowers was not significantly different ($p = 0.82$), similar to allele frequency ($p = 0.56$).

It should be noted that all of the Olympic and World and European Championship medalists in the study sample had at least one R allele for *ACTN3*. There were only two World Championship participants with XX genotype among the elite rowers.

Discussion

ACTN3 gene is the first structural skeletal-muscle gene for which an association between fitness and performance phenotypes has been demonstrated.[7] Evidence for strong *ACTN3* gene influences on elite athletic performance was first reported by Yang et al[2] in 2003.

The possible mechanism underlying the association of the *ACTN3* R577X polymorphism with athletic performance has been discussed in detail by MacArthur and North[6] and MacArthur et al.[20] A few reports have shown that the *ACTN3* RR and RX genotypes are associated with predisposition to power sports and positively correlated with elite strength/power athletes.[2,11–16]

By contrast, there is the hypothesis that the total deficiency of the α-actinin-3 protein may confer some beneficial effect on endurance performance.[2] This hypothesis was taken into consideration by Norman et al,[27] who suggested that individuals with no *ACTN3* expression may be predisposed to developing a higher percentage of type I muscle fibers, benefiting endurance performance. Additionally, Chan et al[28] have reported that the absence of α-actinin-3, as marked by the X allele, promotes a shift in metabolic pathways favoring aerobic performance in a knockout mouse model. Furthermore, the XX *ACTN3* genotype occurs at higher frequency in some groups of elite endurance athletes compared to controls.[2,11,19]

Our results are contrary to the conclusions listed above. The *ACTN3* 577 XX genotype in all rowers was lower compared to the controls ($p = 0.01$). Only six of the 80 (7.5%) investigated rowers had the XX genotype. In the control group, this percentage was 17.65% (36 of 204 rowers).

Our results show that the R allele of the *ACTN3* gene is advantageous in the case of top-level rowers, in whom endurance and excellent isokinetic strength and power are required.[29] It should be noted that all of the Olympic and World and European Championship medalists in the study sample had at least one R allele for *ACTN3*, and 21 of 37 (56.8%) of all top-level rowers were RR homozygotes.

Several studies have confirmed our findings, in which the XX *ACTN3* genotype was also not found to be associated with endurance performance.[22,24–26,30] In addition, 99% of Kenyan elite endurance athletes[23] and 75% of Greek international-level athletes in endurance-based sports[12] had at least one R allele of the *ACTN3* gene. The same conclusion has been reached by Ahmetov et al,[26] who have explained the high percentage of R allele (and its importance) in endurance-orientated athletes by the fact that, 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. This hypothesis is supported by Lucia et al,[31] who have supposed the very high speeds and near maximal intensities at which top endurance-orientated athletes currently perform during the competitively critical phases of the races in which they must effectively sprint for short distances, probably requires the ability to recruit type II fibers, that is, expressing α-actinin-3 protein. Regarding the rowers, these assumptions seem to be confirmed by the "physiological pattern of the rowing competition" proposed by Hagermen,[32] and the fact reported by Ahmetov et al[26] that male rowers with the *ACTN3* 577XX genotype showed the slowest rowing time compared to the 577RR homozygotes, with a time of approximately 1 minute. It is well established that rowers should exhibit excellent isokinetic strength and power.[29] They utilize a unique physiological pattern of race pacing; they begin exertion with a vigorous sprint that places excessive demands on anaerobic metabolism, followed by a severely high aerobic steady-state and a fast finish.[29]

It is worth observing the lack of significant differences in genotype distribution and allele frequency between elite and nonelite rowers. This fact may suggest that the *ACTN3* R577X polymorphism plays an important role in rowing (regardless of the athlete's status) and may be one of the key factors in long-term participation in the sport in general.

Our findings suggest the important influence of *ACTN3* R allele frequency on rowing. Regarding this hypothesis, there is the question of how to explain why two elite rowers (Europe Championships medalists) were 577XX homozygotes. Similar observations have been made regarding power-orientated sports such as the long jump[31] or hammer throwing.[15] In this case, there is a hypothesis that α-actinin-3 deficiency in men may be compensated by androgen hormones.[14] It is also worth remembering that, like other sport phenotypes, endurance performance seems to be multifactorial and an oligogenic trait (e.g., a phenotypic trait produced by two or more genes working together).[19] Norman et al[27] have suspected that the lack of α-actinin-3 in the XX genotype does not compromise muscle function, suggesting that the other isoform, α-actinin-2, may compensate for the lack of α-actinin-3.

In conclusion, our results are contrary to the hypothesis that *ACTN3* 577XX allele may confer some beneficial effect on endurance performance in rowing. On the contrary, the *ACTN3* RX or RR genotypes seem to be a requirement for being an elite rower, however, the identification of *ACTN3* polymorphism as a genetic marker for rowing talent should be interpreted with great caution.

References

1. Bouchard C, An P, Rice T, et al. Familial aggregation of VO_2max response to exercise training: result from the HERITAGE Family Study. *J Appl Physiol*. 1999;87:1003−1008.
2. Yang N, MacArthur DG, Gulbin JP, et al. ACTN3 genotype is associated with human elite athletic performance. *Am J Hum Genet*. 2003;73:627−631.
3. Mills M, Yang N, Weinberger R, et al. Differential expression of the actin-binding proteins, alphaactinin -2 and -3, in different species: implications for the evolution of functional redundancy. *Hum Mol Genet*. 2001;10:1335−1346.
4. North KN, Yang N, Wattanasirichaigoon D, et al. A common nonsense mutation results in alpha-actinin-3 efficiency in the general population. *Nat Genet*. 1999;21:353−354.
5. Suminaga R, Matsuo M, Takeshima Y, et al. Nonsense mutation of the alpha-actinin-3 gene is not associated with dystrophinopathy. *Am J Med Genet*. 2000;92:77−78.
6. MacArthur DG, North KN. ACTN3: a genetic influence on muscle function and athletic performance. *Exerc Sport Sci Rev*. 2007;35:30−34.
7. MacArthur DG, North KN. A gene for speed? The evaluation and function of α-actinin-3. *Bio Essays*. 2004;26:786−795.
8. Blanchard A, Ohanian V, Critchley D. The structure and function of alpha-actinin. *J Muscle Res Cell Motil*. 1989;10:280−289.
9. Beggs AH, Byers TJ, Knoll JH, et al. Cloning and characterization of two human skeletal muscle alpha-actinin genes located on chromosomes 1 and 11. *J Biol Chem*. 1992;267:9281−9288.
10. Vincent B, De Bock K, Ramaekers M, et al. ACTN3 (R577X) genotype is associated with fiber type distribution. *Physiol Genomics*. 2007;32:58−63.
11. Niemi AK, Majamaa K. Mitochondrial DNA and ACTN3 genotypes in Finnish elite endurance and sprint athletes. *Eur J Hum Genet*. 2005;13:965−969.
12. Papadimitriou ID, Papadopoulos C, Kouvatsi A, et al. The ACTN3 gene in elite Greek track and field athletes. *Int J Sports Med*. 2007;29:352−355.
13. Roth SM, Walsh S, Liu D, et al. The ACTN3 R577X nonsense allele is under-represented in elite-level strength athletes. *Eur J Hum Genet*. 2008;16:391−394.
14. Santiago C, González-Freire M, Serratosa L, et al. ACTN3 genotype in professional soccer players. *Br J Sports Med*. 2008;42:71−73.
15. Druzhevskaya AM, Ahmetov II, Astratenkova IV, et al. Association of the ACTN3 R577X polymorphism with power athlete status in Russians. *Eur J Appl Physiol*. 2008;103:631−634.
16. Cieszczyk P, Eider J, Ostanek M, et al. Association of the ACTN3 R577X polymorphism in Polish power-orientated athletes. *J Hum Kinet*. 2011;28:5−13.
17. Clarkson PM, Devaney JM, Gordish-Dressman H, et al. ACTN3 genotype is associated with increases in muscle strength and response to resistance training in women. *J Appl Physiol*. 2005;99:154−163.
18. Delmonico MJ, Kostek MC, Doldo NA, et al. Alpha-actinin-3 (ACTN3) R577X polymorphism influences knee extensor peak power response to strength training in older men and women. *J Gerontol A Biol Sci Med Sci*. 2007;62:206−212.
19. Eynon N, Duarte JA, Oliveira J, et al. ACTN3 R577X polymorphism and Israeli top-level athletes. *Int J Sports Med*. 2009;30:695−698.
20. MacArthur DG, Seto JT, Raftery JM, et al. Loss of ACTN3 gene function alters mouse muscle metabolism and shows evidence of positive selection in humans. *Nat Genet*. 2007;39:1261−1265.
21. MacArthur DG, Seto JT, Chan S, et al. An Actn3 knockout mouse provides mechanistic insights into the association between α-actinin-3 deficiency and human athletic performance. *Hum Mol Genet*. 2008;17:1076−1086.
22. Papiarni A, Ripani M, Giordano GD. ACTN3 genotyping by real-time PCR in the Italian population and athletes. *Med Sci Sports Exerc*. 2007;39:810−815.
23. Yang N, MacArthur D, Wolde B, et al. ACTN3 genotype is not associated with elite endurance athlete status in Ethiopians and Kenyans. *Med Sci Sports Exerc*. 2005;37:472.
24. Santiago C, Ruiz JR, Muniesa CA, et al. Does the polygenic profile determine the potential for becoming a world-class athlete? Insights from the sport of rowing. *Scand J Med Sci Sports*. 2010;20:188−194.
25. Muniesa CA, González-Freire M, Santiago C, et al. World-class performance in lightweight rowing: is it genetically influenced? A comparison with cyclists, runners and non-athletes. *Br J Sports Med*. 2010;44:898−901.
26. Ahmetov II, Druzhevskaya AM, Astratenkova IV, et al. The ACTN3 R577X polymorphism in Russian endurance athletes. *Br J Sports Med*. 2010;44:649−652.
27. Norman B, Esbjörnsson M, Rundqvist H, et al. Strength, power, fiber types and mRNA expression in trained men and women with different ACTN3 R577X genotypes. *J Appl Physiol*. 2009;106:959−965.
28. Chan S, Seto JT, MacArthur DG, et al. A gene for speed: contractile properties of isolated whole EDL muscle from an alpha-actinin-3 knockout mouse. *Am J Physiol*. 2008;295:897−904.
29. Secher NH. Physiological and biomechanical aspects of rowing. Implications for training. *Sports Med*. 1993;15:24−42.
30. Saunders CJ, September AV, Xenophontos SL, et al. No association of the ACTN3 gene R577X polymorphism with endurance performance in Ironman Triathlons. *Ann Hum Genet*. 2007;71:777−781.
31. Lucia A, Gómez-Gallego F, Santiago C, et al. ACTN3 genotype in professional endurance cyclists. *Int J Sports Med*. 2006;27:880−884.
32. Hagerman FC. Applied physiology of rowing. *Sports Med*. 1984;1:303−326.