The ACTN3 R577X Polymorphism in East and West African Athletes

NAN YANG[1], DANIEL G. MACARTHUR[1], BEZABHE WOLDE[2], VINCENT O. ONYWERA[3], MICHAEL K. BOIT[3], SAU YIN MARY-ANN LAU[1], RICHARD H. WILSON[4], ROBERT A. SCOTT[4], YANNIS P. PITSILADIS[4], and KATHRYN NORTH[1,5]

[1]Institute for Neuromuscular Research, the Children's Hospital at Westmead, Sydney, AUSTRALIA; [2]Kotebe College of Teacher Education, Addis Ababa, ETHIOPIA; [3]Department of Exercise and Sports Science, Kenyatta University, Nairobi, KENYA; [4]International Centre for East African Running Science (ICEARS), University of Glasgow, Glasgow, SCOTLAND; and [5]Discipline of Paediatrics and Child Health, Faculty of Medicine, University of Sydney, AUSTRALIA

BASIC SCIENCES

ABSTRACT

YANG, N., D. G. MACARTHUR, B. WOLDE, V. O. ONYWERA, M. K. BOIT, S. Y. M. LAU, R. H. WILSON, R. A. SCOTT, Y. P. PITSILADIS, and K. NORTH. The *ACTN3* R577X Polymorphism in East and West African Athletes. *Med. Sci. Sports Exerc.*, Vol. 39, No. 11, pp. 1985–1988, 2007. **Purpose:** To determine the frequency of the *ACTN3* R577X polymorphism (functional R allele and nonfunctional X allele) in a variety of African populations and to examine its influence on the success of elite East African endurance runners and West African sprinters. **Methods:** The R577X polymorphism was genotyped in 198 Ethiopian controls and 76 elite Ethiopian endurance athletes, 158 Kenyan controls and 284 elite Kenyan endurance runners, and 60 Nigerian controls and 62 elite Nigerian power athletes. Statistical analyses were performed by exact tests of population differentiation, using Arlequin, version 3. Analyses were carried out using 1×10^6 Markov chain steps, and 1×10^5 dememorization steps. **Results:** The frequency of the X allele was extremely low among Kenyans and Nigerians (~1% homozygosity) and higher in Ethiopians (~11% homozygosity). The low baseline frequencies of the three populations tested mean that any associations with sprint performance would likely be obscured. In Ethiopians, where baseline levels of 577XX were about 11%, there was no increased frequency in the endurance athletes. **Conclusion:** Our data suggest that α-actinin-3 deficiency is not a major influence on performance in African athletes. **Key Words:** GENETICS, ACTININ-3, AFRICA, RUNNERS, ATHLETIC PERFORMANCE

The actin-binding protein α-actinin-3, encoded by the *ACTN3* gene, is a highly conserved component of the contractile machinery in fast skeletal muscle fibers in mammals (7). Homozygosity for a common nonsense polymorphism, R577X, results in complete deficiency of α-actinin-3 in about 16% of the global human population (11). We have previously demonstrated a strong association between the R577X polymorphism and elite athlete status in Australian Caucasian populations, with the α-actinin-3–deficient XX genotype being present at a lower frequency in sprint/power athletes, and at slightly higher frequency in elite female endurance athletes, relative to controls (18). The negative association of the XX genotype with sprint performance was subsequently replicated in a cohort of Finnish elite track and field athletes (10). The possible positive association of XX with endurance performance remains intriguing but uncertain, with two recent smaller studies comparing R577X genotype frequencies between 50 elite endurance cyclists, 52 elite endurance runners, and 123 controls from Spain (6), and 42 male rowers and 102 male controls from Italy (13), finding no significant differences in R577X genotype frequencies between control and athlete groups.

R577X genotype has also been associated with muscle function parameters in several large nonathlete cohorts. An analysis of 355 females demonstrated an association between the XX genotype and lower baseline muscle strength (1), whereas a study of older individuals found that R577X genotype influences the response of the quadriceps muscle to strength training (2). In a large cohort of Greek adolescents, the XX genotype was associated with significantly slower performance in a 40-m sprint (9). These data suggest that the presence of α-actinin-3 is required for optimal muscle contraction at high velocity, and that the *ACTN3* R577X polymorphism influences muscle performance in humans. Although the link between R577X and muscle strength and sprint performance is now reasonably well established, the association between the X allele and endurance performance remains tentative.

Address for correspondence: Kathryn North, M.D., FRACP, Associate Dean and Head of Discipline, Paediatrics and Child Health, Faculty of Medicine, University of Sydney, Head, Neurogenetics Research Unit, Deputy Head, Institute for Neuromuscular Research, The Children's Hospital at Westmead, Locked Bag 4001, Westmead, 2145, Sydney, NSW, Australia; E-mail: kathryn@chw.edu.au.
Submitted for publication February 2007.
Accepted for publication June 2007.

0195-9131/07/3911-1985/0
MEDICINE & SCIENCE IN SPORTS & EXERCISE®
Copyright © 2007 by the American College of Sports Medicine
DOI: 10.1249/mss.0b013e31814844c9

Copyright © 2007 by the American College of Sports Medicine. Unauthorized reproduction of this article is prohibited.

All association studies to date have focused on individuals of European ancestry, leaving open the question of whether the effect of α-actinin-3 deficiency on muscle function differs between ethnic groups. East Africans (Ethiopians and Kenyans) dominate modern middle-distance and marathon running competitions. Only 3 of the 40 best-ever 10,000-m running times have been recorded by athletes from countries other than Ethiopia and Kenya (5). Meanwhile, athletes with West African ancestry have emerged as the world's fastest sprinters. It has been speculated that the success of these African athletes has a genetic basis, but this suggestion remains controversial (14). Some supporting evidence has emerged in a recent study showing that some Y chromosomal haplotype groups distribute differentially between endurance runners and controls from Ethiopia (8), although other genetic markers previously associated with performance have failed to show significant associations in African athletes (16,17). Therefore, the purpose of this study was to examine the distribution of R577X genotypes in East and West African populations and its contribution to the success in cohorts of elite distance runners from East Africa (Ethiopia and Kenya) and power athletes from West Africa (Nigeria).

BASIC SCIENCES

MATERIALS AND METHODS

This study was approved by the Glasgow University ethics committee, local Ethiopian, Kenyan, and Nigerian authorities, and the ethics committees of the Children's Hospital at Westmead and the University of Sydney. The East African cohort consisted of 198 Ethiopian controls and 76 elite Ethiopian endurance athletes, all members of the Ethiopian junior or senior athletics squad (15). Among the controls, 105 samples were collected from the general Ethiopian population (15), and another 93 samples were collected from the Arsi province, a region known to be the origin of many elite endurance athletes (15). In addition, 158 Kenyan controls and 284 elite endurance runners were included, 67 of whom had represented Kenya in major international distance running competition, and the remainder of whom were competitive in distance running competition at national level within Kenya. (12). Endurance athletes were competitive in events from 3000 m to marathon. The Nigerian cohort consisted of 60 controls and 62 power athletes, who consisted of track runners up to 400 m, and jump athletes competing in 110-m hurdles and long and triple jumps. Twenty of the above had been international competitors representing Nigeria, and the remainder were competitive at national level. Buccal swab samples were collected, and DNA was extracted as previously described (16). Japanese DNA samples ($N = 97$) were kindly donated by the National Centre of Neurology and Psychiatry Tokyo Japan. The R577X polymorphism was genotyped using Applied Biosystems TaqMan SNP Assay (Cat# C_590093_1). For quality control, about 10% of the samples were also assayed using a *Dde*I restriction fragment-length polymorphism assay, as described (7), and sequenced to confirm the results. Statistical analyses were performed by exact tests of population differentiation, using Arlequin, version 3 (3). Analyses were carried out using 1×10^6 Markov chain steps, and 1×10^5 dememorization steps.

RESULTS

ACTN3 genotype frequencies of Ethiopian, Kenyan, Nigerian, and Japanese subjects are shown alongside previously published frequencies in Table 1. There were no significant deviations from Hardy–Weinberg equilibrium in the populations tested (Table 1). The XX (α-actinin-3 null) genotype is absent in Nigerians, at extremely low frequency in Kenyans (~1%), but higher in Ethiopians (11%) (Table 1). Japanese subjects have a higher frequency of the XX genotype (24%), in line with other Asian populations (7). Results from previous mtDNA analyses suggest that Ethiopians have experienced considerable genetic interaction with neighboring Arabian populations

TABLE 1. Distributions of *ACTN3* R577X polymorphism in African populations and elite athletes, and in other human populations.

	Genotype Frequency No. (%)			Allele Frequency No. (%)		
Subjects	**RR**	**RX**	**XX**	**R**	**X**	**Geographic Origin**
Ethiopians						Northeast Africa
General control (N = 105)	46 (44)	46 (44)	13 (12)	138 (66)	72 (34)	
Arsi control (N = 93)	37 (40)	47 (50)	9 (10)	121 (65)	65 (35)	
Total control (v = 198)	83 (42)	93 (47)	22 (11)	259 (65)	137 (35)	
Endurance runners (N = 76)	35 (46)	35 (46)	6 (8)	105 (69)	47 (31)	
Kenyans*						East Africa
Control (N = 158)	133 (84)	23 (15)	2 (1)	289 (91)	27 (9)	
Endurance runners (N = 284)	212 (75)	69 (24)	3 (1)	493 (87)	75 (13)	
Nigerians						Northwest Africa
Control (N = 60)	50 (83)	10 (17)	0 (0)	110 (92)	10 (8)	
Power athletes (N = 62)	54 (87)	8 (13)	0 (0)	116 (94)	8 (6)	
South African Bantu# (7) (N = 88)	69 (78)	18 (21)	1 (1)	156 (89)	20 (11)	South Africa
Australian Caucasian# (18) (N = 436)	130 (30)	226 (52)	80 (18)	486 (56)	386 (44)	Europe
Spanish# (6) (N = 123)	35 (28)	66 (54)	22 (18)	136 (55)	110 (45)	Europe
Japanese (N = 97)	17 (17)	57 (59)	23 (24)	91 (47)	103 (53)	Northeast Asia
Javanese# (7) (N = 48)	8 (17)	28 (58)	12 (25)	44 (46)	52 (54)	Southeast Asia
PNG Highlander# (7) (N = 39)	17 (44)	16 (41)	6 (15)	50 (64)	28 (36)	Oceania
Aboriginal Australian# (7) (N = 87)	45 (52)	33 (38)	9 (10)	123 (71)	51 (29)	Australia

* Genotype distributions between Kenyan control and Kenyan endurance runners are significantly different (P = 0.036); # genotype frequencies were published previously in cited references.

Copyright © 2007 by the American College of Sports Medicine. Unauthorized reproduction of this article is prohibited.

in recent time (4), so gene flow from non-African populations may explain the higher frequency of the X allele in Ethiopians relative to Kenyans.

Ethiopian endurance athletes did not differ from general controls (P = 0.69), nor from controls from the Arsi region (P = 0.72). Kenyan endurance athletes did differ significantly from the Kenyan control group (P = 0.036) (Table 1). When the most successful Kenyan athletes were compared with controls, there was a tendency toward differences in genotype frequency (P = 0.063), which did not reach significance. However, there is no increase in the frequency of XX genotypes in the endurance athletes to suggest an advantage to endurance performance (controls: 1%; endurance: 1%); rather, it is an excess of heterozygotes (24%), or deficiency of R homozygotes, in the endurance athletes relative to controls (15%) that elicits the significant differences between groups (Table 1). Previous reports have suggested that the influence of the X allele on endurance performance may be stronger in females (18); however, significant differences were attenuated when only female Kenyan athletes and controls were compared (P = 0.09).

No differences were present between Nigerian control subjects and Nigerian power athletes (P = 0.62). However, given that sprint/power athlete status is associated with a lower frequency of the XX genotype than controls in previous studies of European athletes, the complete absence of the XX genotype in the general Nigerian population eliminates the possibility of a similar association between sprint ability and *ACTN3* genotype in Nigerians.

DISCUSSION

The X allele has been observed in all human populations tested so far. The lowest X allele frequencies occur in Kenyan, Nigerian and South African populations (8–11%), resulting in approximately 1% XX genotype frequency. Australian Caucasian and Spanish with European origin, Japanese in northeast Asia, and Javanese in southeast Asia have the highest frequencies of the X allele (44–54%), resulting in XX genotype frequencies from 18 to 25%. The X allele frequencies in the Japanese and Javanese populations are higher than 50%. Papua New Guinea highlanders and Aboriginal Australians display intermediate X allele frequencies (29–36%), resulting in XX genotype frequencies of 10–15% (11).

This study does not support the hypothesis that the *ACTN3* null X allele contributes to the success of the East African endurance runners relative to their source populations. In addition, the extremely low XX genotype frequency in the Nigerian control population (0% in this study) rules out the possibility of detecting a lower frequency of the XX genotype in Nigerian power athletes.

There are several possible explanations for the discrepancy between these results and previously published associations seen in athletes of European descent. The low baseline allele frequency of 577X in the three surveyed African populations reduces the effective power of our study, potentially obscuring any genetic association with performance. Alternately, it is possible that the effect of *ACTN3* genotype on muscle performance is dependent on other genetic influences, or on environmental variables, that differ between African and non-African populations. For example, many East Africans are subject to environmental influences, such as living and training at altitude and high levels of incidental running during childhood, which play major roles in shaping athletes from this region (12,15) and differ substantially from the childhood experiences of most potential non-African athletes. Such factors may reduce the impact of α-actinin-3 deficiency on the muscle performance of African individuals.

In conclusion, the results of this study extend our knowledge of the distribution of the 577X null allele of the *ACTN3* gene to three previously unexamined African populations. We have shown that the frequency of the 577X allele is substantially lower in Kenyan and Nigerian populations than in any non-African population sampled to date, and it is similar to that seen previously in a South African Bantu-speaking cohort (Table 1) (7). The 577X allele frequency seen in the Ethiopian group is substantially higher, probably reflecting population admixture with non-African groups. We have found no evidence for an association between the R577X polymorphism and endurance performance in East African athletes. The absence of 577XX (α-actinin-3 deficiency) in controls and athletes from Nigeria would mask any association between the presence of α-actinin-3 and sprint/power performance. Our data suggest that α-actinin-3 deficiency is not a major influence on performance in African athletes.

Financial support for this study was partly provided by grant (#301905) from NHMRC Australia to K. North and N. Yang.

BASIC SCIENCES

REFERENCES

1. Clarkson, P. M., J. M. Devaney, H. Gordish-Dressman, et al. ACTN3 genotype is associated with increases in muscle strength in response to resistance training in women. *J. Appl. Physiol.* 99: 154–163, 2005.
2. Delmonico, M. J., M. C. Kostek, N. A. Doldo, et al. α-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.* 62:206–212, 2007.
3. Excoffier, L., G. Laval, and S. Schneider. Arlequin ver. 3.0: an integrated software package for population genetics data analysis. *Evol. Bioinform. Online* 1:47–50, 2005.
4. Kivisild, T., M. Reidla, E. Metspalu, et al. Ethiopian mitochondrial DNA heritage: tracking gene flow across and around the gate of tears. *Am. J. Hum. Genet.* 75:752–770, 2004.
5. Larsen, H. B. Kenyan dominance in distance running. *Comp. Biochem. Physiol. Part A.* 136:161–170, 2003.

Copyright © 2007 by the American College of Sports Medicine. Unauthorized reproduction of this article is prohibited.

6. Lucia, A., F. Gomez-Gallego, C. Santiago, et al. ACTN3 genotype in professional endurance cyclists. *Int. J. Sports Med.* 27:880–884, 2006.
7. Mills, M., N. Yang, R. Weinberger, et al. Differential expression of the actin-binding proteins, alpha-actinin-2 and -3, in different species: implications for the evolution of functional redundancy. *Hum. Mol. Genet.* 10:1335–1346, 2001.
8. Moran, C. N., R. A. Scott, S. M. Adams, et al. Y chromosome haplogroups of elite Ethiopian endurance runners. *Hum. Genet.* 115:492–497, 2004.
9. Moran, C. N., N. Yang, M. E. Bailey, et al. Association analysis of the ACTN3 R577X polymorphism and complex quantitative body composition and performance phenotypes in adolescent Greeks. *Eur. J. Hum. Genet.* 15:88–93, 2007.
10. Niemi, A. K., and K. Majamaa. Mitochondrial DNA and ACTN3 genotypes in Finnish elite endurance and sprint athletes. *Eur. J. Hum. Genet.* 13:965–969, 2005.
11. North, K. N., N. Yang, D. Wattanasirichaigoon, M. Mills, S. Easteal, and A. H. Beggs. A common nonsense mutation results in alpha-actinin-3 deficiency in the general population. *Nat. Genet.* 21:353–354, 1999.
12. Onywera, V. O., R. A. Scott, M. K. Boit, and Y. P. Pitsiladis. Demographic characteristics of elite Kenyan endurance runners. *J. Sports Sci.* 24:415–422, 2006.
13. Paparini, A., M. Ripani, G. D. Giordano, D. Santoni, F. Pigozzi, and V. Romano-Spica. ACTN3 genotyping by real-time PCR in the Italian population and athletes. *Med. Sci. Sports Exerc.* 39:810–815, 2007.
14. Pitsiladis, Y. P., and R. A. Scott. Essay: the makings of the perfect athlete. *Lancet* 366(Suppl. 1):S16–S17, 2005.
15. Scott, R. A., E. Georgiades, R. H. Wilson, W. H. Goodwin, B. Wolde, and Y. P. Pitsiladis. Demographic characteristics of elite Ethiopian endurance runners. *Med. Sci. Sports Exerc.* 35:1727–1732, 2003.
16. Scott, R. A., C. N. Moran, R. H. Wilson, et al. No association between angiotensin converting enzyme (ACE) gene variation and endurance athlete status in Kenyans. *Comp. Biochem. Physiol. Part A.* 141:169–175, 2005.
17. Scott, R. A., R. H. Wilson, W. H. Goodwin, et al. Mitochondrial DNA lineages of elite Ethiopian athletes. *Com. Biochem. Physiol. Part B.* 140:497–503, 2005.
18. Yang, N., D. G. MacArthur, J. P. Gulbin, et al. ACTN3 genotype is associated with human elite athletic performance. *Am. J. Hum. Genet.* 73:627–631, 2003.

Copyright © 2007 by the American College of Sports Medicine. Unauthorized reproduction of this article is prohibited.