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ACTN3: A Genetic Influence on Muscle Function and Athletic Performance

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MACARTHUR, D.G. and K.N. NORTH. *ACTN3*: A genetic influence on muscle function and athletic performance. *Exerc. Sport Sci. Rev.*, Vol. 35, No. 1, pp. 30–34, 2007. *A common variant of the ACTN3 gene, R577X, results in complete deficiency of the α-actinin-3 protein in the fast skeletal muscle fibers of more than a billion humans worldwide. We review the evidence that this genetic variant is strongly associated with elite athlete status and with normal variation in human muscle strength and sprinting speed.*
Key Words: R577X, actinin, genetic association, elite athletes, muscle strength

INTRODUCTION

Athletic performance is a complex human trait influenced by environmental parameters such as diet, training, opportunity, and heritable factors, that is, genetic makeup. Genetic factors determine 20%–80% of the variation in a wide variety of traits relevant to athletic performance, such as oxygen uptake (2), cardiac output (1), and the relative proportion of fast and slow fibers in skeletal muscle (14). For the last decade, researchers have identified a large number of the individual genes underlying these influences, an effort highlighted in the annual publication of the human gene map for performance and health-related fitness phenotypes. The latest edition of this human gene map (15) lists more than 150 genes or genetic regions associated with athletic performance and physical fitness traits.

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 contractile (sarcomeric) apparatus in the fast glycolytic fibers of human skeletal muscle—the fibers responsible for the generation of rapid, forceful contractions in activities such as sprinting and weightlifting—and is thought to perform specialized role(s) important to the function of these fibers. The precise functions of α-actinin-3 are still unknown but are likely to include a structural role in the maintenance of muscle mechanical integrity and possibly other functions related to muscle signaling and metabolism (8).

Several years ago, our team identified a common genetic variation in the *ACTN3* gene that results in the replacement of an arginine (R) with a stop codon (X) at amino acid 577 (R577X). This variation creates two different versions of the *ACTN3* gene, both of which are common in the general population: the 577R version (or 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 (12).

Because every human inherits two copies of the *ACTN3* gene—one maternal and one paternal copy—there are three possible combinations or genotypes of R577X alleles. In individuals of European descent, less than a third of the population have two copies of the functional R allele (the RR genotype), whereas just over half the population have one copy of each of the two alleles (the RX genotype). Remarkably, the remaining 18% of the healthy European population—and more than a billion people worldwide—have two copies of the nonfunctional 577X variant (the XX genotype), resulting in complete deficiency of α-actinin-3 protein in their skeletal muscle (9).

This widespread deficiency of a potentially important skeletal muscle protein is extremely unusual. The fact that more than a billion humans lack this protein without experiencing overt muscle disease suggests that α-actinin-3 is at least partially redundant and that many of its functions can be compensated for by other factors, most likely including the closely related protein α-actinin-2. However, the specialized expression pattern and strong sequence conservation of α-actinin-3 over more than 300 million years of evolutionary time (9) suggest that this protein does possess some roles in fast fibers that cannot be completely

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taken over by α-actinin-2. If so, we reasoned, the absence of α-actinin-3 in the skeletal muscle is likely to have an effect on fast muscle fibers and may contribute to variation in muscle function in the general population.

To study this possibility, we and other groups have performed genetic association studies on the R577X polymorphism. These studies use two major approaches: case-control analysis of elite athletes and cross-sectional analysis of nonathletes. In case-control studies, R577X genotypes are collected from groups of elite athletes, and the frequency of each genotype in these groups are compared with the frequency in a large population of sedentary (nonelite) controls. If one genotype is more favorable to elite athletic performance than the other genotypes, it will be found at a higher frequency in elite athletes than in controls. In cross-sectional association studies, large groups of individuals are tested for one or more traits (such as muscle strength or sprint speed), and then the group is broken down by R577X genotype. If one genotype is beneficial for the studied trait, individuals with that genotype will tend to have higher values for that trait than individuals with other genotypes.

CASE-CONTROL ASSOCIATION STUDIES IN ELITE ATHLETES

The first association study to explore the influence of *ACTN3* genotype on athletic performance was published by our team (16). We studied DNA samples from a large group of elite athletes from the Australian Institute of Sport competing in a wide variety of sports, along with a cohort of 436 nonathlete controls drawn from the general population. All athletes and controls in this study were of European ancestry because we have also shown that the frequency of the R577X polymorphism varies in different ethnic groups (9). We hypothesized that α-actinin-3 deficiency would predominantly affect the function of fast muscle fibers and thus have a greater influence on athletes competing in sprint or power events who specifically require optimal fast-fiber performance, rather than on athletes competing in endurance events who rely predominantly on slow muscle fibers. We thus asked the Australian Institute of Sport to classify the athletes based on their specialized area of performance: the final cohort consisted of a group of 107 athletes competing in sprint or power events, such as short-distance running, swimming, and cycling, and a group of 194 athletes competing in endurance events, such as long-distance running, cycling, and cross-country skiing. We then determined the R577X genotype for each of the DNA samples to allow comparison of the frequency of α-actinin-3 deficiency between athlete groups and controls.

The results of this analysis (16) are shown in Figure 1. The frequency of the α-actinin-3–deficient XX genotype was almost 20% in our control cohort, similar to the levels previously determined in individuals of European ancestry (9). In power athletes, this frequency was dramatically reduced: in the power athlete cohort as a whole, the frequency of α-actinin-3 deficiency was only 6%, about a third of the levels in controls, whereas none of the Olympians or female power athletes had an XX (α-actinin-3–deficient) genotype. This reduction in frequency was specific to power athletes; if anything, endurance athletes showed a slight increase in the frequency of α-actinin-3 deficiency, although this was only significant in females.

These data suggested that the presence of α-actinin-3 is required for optimal fast-fiber performance in power athletes, whereas the absence of α-actinin-3 may provide some sort of advantage for endurance athletes. The R577X polymorphism thus joined the growing list of genetic factors reported to influence athletic performance (15).

The association between R577X and athletic performance certainly has biological plausibility: the R577X polymorphism has a clear biochemical effect, completely eliminating the production of functional α-actinin-3 protein; in addition, the localization of α-actinin-3 to fast muscle fibers is consistent with a negative effect of α-actinin-3 deficiency on sprint/power performance.

Nevertheless, an isolated genetic association study must be treated with caution because there are numerous ways in which such studies can generate false-positive results (5,13). The association of R577X with athletic performance has now been supported by the independent replication of our findings in a study of elite Finnish athletes (11). This study compared the frequency of α-actinin-3 deficiency in a group of 68 sprint athletes, 40 endurance athletes, and 120 ethnically matched controls and found a very similar pattern to our own study of Australian athletes: a marked decrease in the frequency of α-actinin-3 deficiency in sprint athletes and a slight (but not significant) increase in the frequency of the XX genotype in endurance athletes. In both cases, these trends were most apparent in athletes who had competed at an international level.

These two studies, taken together, provide reasonably solid evidence for the notion that the presence of α-actinin-3 is important for the optimal performance of fast fibers in

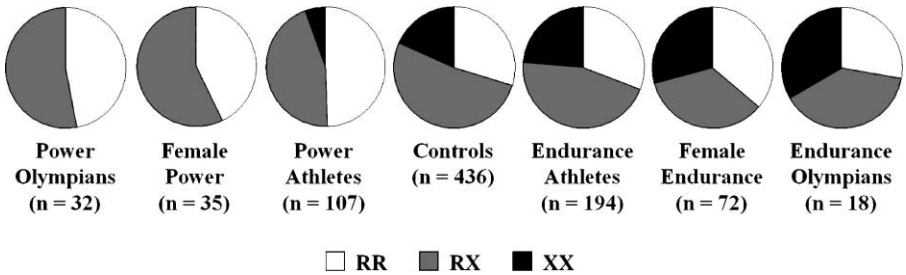

Figure 1. R577X genotype frequencies in controls and elite sprint and endurance athletes from Yang *et al.* (16). The frequency of the 577XX (α-actinin-3–deficient) genotype is significantly lower in the total power athlete group (6%) than in controls (18%) and significantly higher in female endurance athletes (29%) than in female controls. The power Olympian and female power athlete groups both contain no 577XX individuals.

sprint/power activities. However, the possible positive association of α-actinin-3 deficiency with endurance athletes seen in our study (16) is not statistically significant in the Finnish study. In addition, a more recent study (6) comparing 50 elite male endurance cyclists and 52 Olympic-level endurance runners with 123 sedentary male controls found no significant differences in genotype frequencies between controls and either of the two athlete groups (although the frequency of α-actinin-3 deficiency was slightly higher in the endurance cyclist cohort than in controls, 26% vs 17.9%). There was also no association between R577X genotype and a common measure of endurance performance—maximal oxygen uptake (VO_2max)—in either of the athlete groups.

CROSS-SECTIONAL ASSOCIATION STUDIES IN NONATHLETE COHORTS

Since our initial publication (16), several cross-sectional association studies have examined the correlation of R577X genotype with a variety of measures of muscle function in nonathlete populations. These studies have provided evidence that the effect of α-actinin-3 deficiency on performance is not restricted to elite athletes and that R577X is one of the many genetic factors that influence variation in muscle function in the general population.

The first study to assess the association of R577X with muscle function in nonathletes examined elbow flexor strength, both at baseline and after a 12-wk resistance-training protocol, in a large cohort of 247 men and 355 women aged 18–40 yr (3). Isometric strength was estimated using a measure of maximal voluntary contraction, whereas dynamic strength was measured using a one repetition maximum protocol. The α-actinin-3–deficient (577XX) genotype was associated with significantly lower baseline maximal voluntary contraction in women (but not in men); unexpectedly, α-actinin-3–deficient women also showed a significantly greater response to training for the one repetition maximum measurement. These associations were significant in both the European and Asian subsets of the female cohort.

The authors argue that their results are consistent with a role for α-actinin-3 in the maintenance of sarcomeric integrity, which fits with the known interactions of the α-actinins with a variety of structural proteins (8). Greater exercise-induced muscle damage in the absence of α-actinin-3 may result in poorer overall power generation, but it may also stimulate adaptive remodeling of the sarcomere, resulting in a more rapid response to training. This explanation is speculative, and further studies will be needed both to confirm the positive effect of α-actinin-3 deficiency on the response to strength training and to dissect out the mechanisms underlying this effect.

Another study by the same group assessed the effect of the R577X polymorphism on an indirect measure of muscle damage (4). In this study, 78 men and 79 women with a mean age of 24 yr performed 50 maximal eccentric contractions of the elbow flexor muscles, a protocol designed to produce subclinical muscle damage. Serum creatine kinase (CK), a well-characterized marker of muscle membrane disruption, was assayed both at baseline and at various time points after the exercise protocol. No association was seen between R577X genotype and the change in serum CK levels after exercise-induced injury. Unexpectedly, there was a (weakly significant) lower level of baseline serum CK levels in α-actinin-3–deficient individuals compared with RR and RX subjects; the authors speculated that α-actinin-3 deficiency may be associated with lower baseline muscle mass or physical activity levels. Given the small sample size and the weakness of the association, this finding should be regarded as tentative, but certainly warrants further study.

The largest study to date on the association of *ACTN3* with muscle performance traits examined R577X genotype in 525 adolescent boys and 467 adolescent girls aged between 11 and 18 yr (10). These individuals had previously been examined for a variety of traits related to body composition, strength/power, and endurance performance. The authors showed a significant association between R577X genotype and performance in a 40-m sprint, with XX (α-actinin-3–deficient) adolescent boys—but not adolescent girls—taking significantly longer to run the distance compared with their RR or RX counterparts (Fig. 2). Interestingly, this association was highly specific: no association was found between R577X genotype and other strength/power phenotypes (such as handgrip strength, basketball throw, and vertical jump height), or with a proxy measure for aerobic capacity, the shuttle run test. In addition, R577X genotype did not correlate significantly with measures of body composition, such as body mass index and skinfold widths. The authors argue that the specific association of R577X genotype with sprint performance is consistent with a primary role for α-actinin-3 in the protection of the sarcomere from mechanical damage during repetitive force generation because sprinting requires repeated cycles of muscle contraction, unlike the other strength/power phenotypes tested (such as vertical jump), which require only a single, forceful muscle contraction.

The male-specific association between R577X and sprint performance observed by Moran *et al.* (10) contrasts with the stronger female associations seen in our athlete study (16) and in the nonathlete cohort studied by Clarkson *et al.* (3). We have previously suggested that the apparent differential effect of R577X genotype on performance in females and males may relate to differences in the relative influence

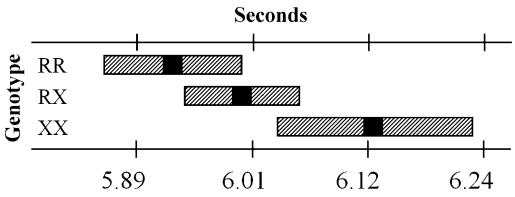

Figure 2. R577X genotype is associated with 40-m sprint times in adolescent boys. Mean and SD (*black boxes*) and 95% confidence intervals (*hatched bars*) for 40-m sprint times (seconds) are shown for each of the three R577X genotypes. The α-actinin-3–deficient 577XX genotype is associated with longer sprint times than the 577RR or 577RX genotypes. [Adapted from Moran, C.N., N. Yang, M.E.S. Bailey, A. Tsiokanos, A. Jamurtas, D.G. MacArthur, K.N. North, Y.P. Pitsiladis, and R.H. Wilson. Association analysis of the *ACTN3* R577X polymorphism and complex quantitative body composition and performance phenotypes in adolescent Greeks. *Eur. J. Human Genet.* (in press). Copyright ® 2006 Colin Moran. Used with permission.]

of endogenous steroid hormones on muscle performance between the sexes (16). If so, we would expect this effect to be more pronounced in adults, and the contrasting effect seen by Moran *et al.* (10) may be caused by the fact that their cohort is largely peripubescent.

CONCLUSION

Our understanding of the genetic influences on human physical performance is evolving rapidly in the postgenomic era. This review has presented a recent but growing body of evidence that one particular genetic polymorphism—the R577X variation in the *ACTN3* gene—represents a common genetic influence on athletic performance and skeletal muscle function. However, much more work remains to be done in this area to answer a number of major questions. First, what areas of human physical performance (in both athletes and nonathletes) are most strongly influenced by R577X? Second, exactly how much predictive power does R577X genotype provide in identifying athletic potential? Third, precisely how does α-actinin-3 deficiency affect the function of skeletal muscle? And finally, from a biomedical perspective, what role does this variation play in influencing human health, fitness, and disease?

There is one clear message from the genetic association data presented in this review: it is extremely likely that α-actinin-3 deficiency reduces, in some way, the performance of fast skeletal muscle fibers in both elite athletes and in the nonathlete population. A negative association between the α-actinin-3–deficient XX genotype and elite sprint athlete status has now been observed in two separate studies (11,16), whereas two large independent studies have found that the XX genotype is associated with lower baseline muscle strength (3) and with poorer sprint performance (10) in nonathlete cohorts. All four of these studies support the notion that α-actinin-3 deficiency inhibits the performance of the fast glycolytic muscle fibers responsible for rapid, forceful contraction, making this one of the more consistently supported associations with a broad physical performance trait for any genetic variant.

However, there are also more tentative associations between R577X and other muscle traits that warrant further detailed investigation. In particular, it seems that α-actinin-3 deficiency may have a positive influence on endurance performance, although this has so far been significant in only one study (16). Notably, in this study, the association of R577X with endurance performance was strikingly sex-specific, being significant only in female subjects. The sex breakdown of the Finnish athlete cohort (11) is unclear, and Lucia *et al.* (6) studied only male athletes, so we would argue that further studies targeted toward female endurance athletes would be worthwhile. In addition, a single study has suggested that α-actinin-3 deficiency might boost the response of muscle to training (3). Further large genetic association studies, involving both elite athletes from a wider variety of sports and nonathletes examined for a broader range of performance traits, will be required to investigate these areas further.

Once the influence of R577X on athletic performance and muscle function has been clearly elucidated, this information may be useful for athlete talent identification programs. Indeed, a biotechnology company, Genetic Technologies, is already marketing an *ACTN3* gene test: in exchange for a simple cheek swab to provide DNA and a check for approximately $85, this company offers to send back an R577X genotype result. Given the growing support described above for the notion that R577X influences muscle function, this intuitively seems like a test that may be useful for coaches and sporting bodies or for young hopefuls deciding whether to pursue a career as an elite athlete—but how useful will R577X genotype information really be in making these decisions?

The answer to this question is still unclear for a number of reasons. First, many different genetic and environmental factors influence physical performance, with R577X genotype determining only a small proportion of overall variation. The cross-sectional association studies cited above estimate that R577X accounts for only 2.2% of the total variance in baseline muscle strength in adult women (3) and 2.6% of the total variance in 40-m sprint speed in adolescent boys (10). These values are fairly rough estimates because of the small sample sizes in these studies, and these proportions are likely to be substantially higher in elite athletes because a reduction of environmental variance in this group will increase the relative importance of genetic influences. Nonetheless, these figures emphasize that R577X is just one of a myriad of complex, interacting factors that influence muscle performance. Second, it is uncertain whether R577X genotype actually adds any further information to existing tests used in talent identification. Although this genetic variant does seem to influence skeletal muscle function, it may well be that existing direct tests of muscle power—such as vertical jump, dynamometry, and sprint tests—already capture this information. Sporting bodies and young athletes should thus await the results of further research before using any genetic information to guide decisions about talent identification or sports selection.

Another area in which considerable uncertainty exists is the mechanistic basis for the effect of α-actinin-3 deficiency on muscle function. Although much research has been published on the functions of the α-actinin protein family in general, comparatively little is known about the specific role of α-actinin-3 in skeletal muscle (8). Our group is currently using a number of different experimental approaches to resolve this knowledge gap, including the generation of a knockout mouse model of α-actinin-3 deficiency. We hope to provide insights into the functions of α-actinin-3 that can then be used to guide further research into the influence of the R577X polymorphism on human muscle, an area that is currently being investigated by several groups worldwide.

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