No Association of the ACTN3 Gene R577X Polymorphism with Endurance Performance in Ironman Triathlons

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Summary

Alpha-actinins are major structural components of the Z-discs in skeletal muscle. Alpha-actinin 3 is encoded by the ACTN3 gene and is expressed only in type II muscle fibres. Homozygosity for the nonsense mutation, 577X, within ACTN3 results in deficiency of α-actinin-3 but does not result in an abnormal muscular phenotype. Previous research has found an association of the 577R allele with sprinting and/or power performance. It has also been suggested that the 577X allele may confer an advantage during endurance events. Four hundred and fifty seven Caucasian male triathletes who completed either the 2000 and/or 2001 226 km South African Ironman Triathlons, and 143 Caucasian controls, were genotyped for the R577X mutation within the ACTN3 gene. There were no significant differences in either the genotype (P = 0.486) or allele (P = 0.375) frequencies within the fastest, middle of the field or slowest Caucasian male finishers and the control population. In conclusion, the R577X polymorphism within the ACTN3 gene was not associated with ultra-endurance performance in the 2000 and 2001 South African Ironman Triathlons.

Keywords: alpha-actinins, athletic ability, skeletal muscle

Introduction

Alpha-actinins are a family of actin binding proteins which are major structural components of the sarcomeric Z-discs in skeletal muscle (North et al. 1999). They are believed to be responsible for anchoring the actin-containing filaments, and maintenance of the spatial arrangement of thin filaments within the sarcomere, as well as the interaction of the cytoskeleton with the sarcolemma (Mills et al. 2001). One of the α-actinin isoforms, α-actinin-3, is expressed solely in type II (fast twitch) muscle fibres (North & Beggs, 1996) which are the predominant muscle fibres involved in sprint and power activities. North et al. previously identified a 1747C>T transition within exon 16 of the α-actinin-3 (ACTN3) gene, which results in the R577X mutation (North et al. 1999). Homozygosity for the nonsense mutation, 577X, within ACTN3 results in the deficiency of α-actinin-3 but does not result in an abnormal muscular phenotype. This suggests that this protein is functionally redundant in humans (North et al. 1999). The finding that approximately 16% of the world population has a congenital deficiency of α-actinin-3 (North et al. 1999) further corroborates this theory. Since this gene has been conserved through evolution (North et al. 1999; North & Beggs, 1996), it must fulfil an as yet unidentified function.

Some researchers have suggested that this seemingly redundant protein becomes important under stressful conditions such as, for example, in elite athletic performance. Yang et al. (2003) recently genotyped 429 elite athletes from different sporting disciplines for the R577X mutation within ACTN3, and suggested that the 577R allele confers an advantage in power or sprint events. They reported a significant variation in allele distribution between elite sprint athletes and the control population, as the sprint athletes had a lower frequency of the 577X allele and 577XX genotype. In addition, none of the 35 female elite sprint athletes
in the cohort had the 577XX genotype. Given the apparent advantage of the 577R allele in power or sprint events, it becomes important to address the high frequency of the 577XX ACTN3 genotype in the general population. Yang et al.’s model for the evolutionary conservation of the 577X allele proposes that both alleles confer a selective advantage under different conditions, and are therefore both acted upon by positive selection. In this model the 577R allele of ACTN3 becomes advantageous during elite power and sprint activities while the 577X allele should be advantageous to endurance athletes (Yang et al. 2003).

The Australian company Genetic Technologies, Inc. have used these results to develop the first commercial genetic test for athletic ability (Genetic Technologies 2006). The ACTN3 Sports Gene Test® brochure claims that “for the first time a fast, simple and painless genetic test can identify whether you may be naturally geared toward sprint/power events or towards endurance sporting ability”. The ethical issues arising as a result of this have recently been discussed by Savulescu & Foddy (2005). There is evidence to suggest that the 577XX variant may be associated with performance in endurance track and field events ranging from 800 m to a 42.2 km marathon (Niemi & Majamaa, 2005). However, neither the genotype nor allele frequencies of this polymorphism have been found to be associated with performance in ultra-endurance events. Lucia et al. (2006) failed to find any association between the ACTN3 R577X genotype and performance in Olympic-class endurance runners and professional endurance cyclists. Likewise, Moran et al. (2006) recently reported that this polymorphism was not associated with an endurance phenotype. The aim of this study was, therefore, to determine if there was an association between the R577X genotype within the ACTN3 gene and ultra-endurance performance in the 226 km South African Ironman Triathlons.

Methods

Subjects

Four hundred and fifty seven of the 701 Caucasian male triathletes who completed either the 2000 (272 finishers) and/or the 2001 (544 finishers) South African Ironman Triathlons, of which 115 completed both events, were recruited for this study (Sharwood et al. 2004). In addition, 143 apparently healthy Caucasian male control subjects (Con) that had not participated in or trained for an ultra-endurance event were recruited from the greater Cape Town Metropolitan area. The triathletes were divided into tertiles based on their overall performance in the triathlons, with the fastest triathletes making up the Fast Triath group, the triathletes finishing in the middle of the field making up the Mid Triath group, and the slowest triathletes making up the Slow Triath group. Both triathlons, held outside Cape Town, were multi-phase endurance events consisting consecutively of a 3.8 km swim, a 180 km cycle and a 42.2 km run. Approval for this study was obtained from the Research and Ethics Committee of the Faculty of Health Sciences, University of Cape Town. The study adhered to the principles of the Declaration of Helsinki.

DNA Extraction and Genotyping

Approximately 4.5 ml of venous blood was collected from each subject into an EDTA vacutainer tube by venupuncture of a fore-arm vein. These samples were stored at 4°C until DNA extraction was performed as described by Lahiri & Nurnberger (1991). The extracted DNA was stored at 4°C until subsequent genotyping. The triathletes were genotyped for the R577X variant of the ACTN3 gene using previously described methods with minor modifications (Mills et al. 2001). Briefly, the DNA sequence was amplified using the following forward, 5′-CTGTGGCTTGATGGAAGG-3′, and reverse, 5′-TGTCACAGTATGCAAGGAGG-3′, primers. The PCR conditions consisted of an initial 5 min denaturing step at 94°C, followed by 35 cycles of denaturing for 30 s at 94°C and annealing for 60 s at 70°C, and a final extension at 72°C for 10 min. The 290 bp amplicon was digested with Ddel and the products separated on a 4% agarose gel. The 577R allele (absence of Ddel site) resulted in fragments of 85 bp and 205 bp, whilst the 577X allele (presence of Ddel site) resulted in fragments of 85 bp, 97 bp and 108 bp.

Statistical Analysis

Data were analysed with the STATISTICA version 7.0 (StatSoft Inc., Tulsa, OK, USA) and GraphPad InStat version 2.05a (GraphPad Software, San Diego, CA, USA) statistical programmes. Differences in genotype frequencies between the triathlete groups were determined by Pearson chi-square (χ²) analysis. The χ² test for linear trend was determined using the GraphPad InStat software. Any significant differences between the characteristics of the triathlete groups were determined by a one-way analysis of variance (ANOVA). When the overall F-value was significant, a Tukey’s honest significance post hoc test was used to determine specific differences. Statistical significance was accepted when P < 0.05. Hardy-Weinberg equilibrium was established using the Genepop web version 3.4 program (http://wbiomed.curtin.edu.au/genepop/).

Results

Subject Characteristics

Seven hundred and one male triathletes completed either the 2000 and/or 2001 South African Ironman Triathlons. Genotype data were obtained from 457 of the Caucasian male triathletes, who were representative of the entire field of triathletes (Saunders et al. 2006). The triathletes were
divided into tertiles based on their overall performance in the 2000 and/or 2001 South African Ironman Triathlons as previously described (Saunders et al. 2006).

As seen in Table 1, the groups were equally matched for height but not for age, weight, BMI or percentage South African born (P < 0.001). The Con group was significantly younger than all three triathlete groups (P < 0.001), and the Fast Triathlete group was significantly younger than the Slow Triathlete group (P = 0.005). The Fast Triathlete group also weighed significantly less than all other groups while the control population weighed the most. Similarly the Fast Triathlete group had the lowest BMI and the control population (P = 0.74) were in Hardy-Weinberg equilibrium.

It should be noted that within the consenting triathletes completing either the 2000 or 2001 event in the 10 fastest overall times (range 521–583 min), three triathletes had the 577XX genotype, six had the 577RR genotype, and one was heterozygous for the R577X polymorphism within ACTN3.

### Genotype and Allele Distributions

Figure 1 shows the ACTN3 R577X genotype (A) and allele (B) frequencies within the three triathlete groups and the Caucasian male control population. There were no significant differences in either the genotype (P = 0.486) or allele (P = 0.375) frequencies within the fastest, middle of the field or slowest finishers and the control population. In addition, there was no significant linear trend for allele distribution between the four groups (P = 0.091). Similar results were obtained when only the South African born athletes were included in the analysis (data not shown).

Both the triathlete group as a whole (P = 0.06) and the control population (P = 0.74) were in Hardy-Weinberg equilibrium.

### Genotype Effects on Performance and Physiological Characteristics

There were no genotype effects on any of the physiological variables recorded nor the split and overall performance times of the triathletes during the event (Table 2). Similar results were obtained when only the South African born athletes were included in the analysis (data not shown).

### Discussion

The aim of this study was to test the association of the ACTN3 R577X polymorphism with performance in the

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**Table 1** General physiological characteristics of the fastest (Fast Triath), middle of the field (Mid Triath) and slowest (Slow Triath) finishing triathlete groups, as well as the control (Con) group.

<table>
<thead>
<tr>
<th></th>
<th>Fast Triath (n = 152)</th>
<th>Mid Triath (n = 152)</th>
<th>Slow Triath (n = 153)</th>
<th>Con (n = 143)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>33.0 ± 6.0 (152)^a,d</td>
<td>33.8 ± 7.5 (152)^b</td>
<td>36.2 ± 9.6 (153)^c,d</td>
<td>28.4 ± 10.0 (141)^a,b,c</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>180.6 ± 6.3 (139)</td>
<td>180.3 ± 6.2 (130)</td>
<td>180.9 ± 7.5 (144)</td>
<td>180.8 ± 8.0 (140)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>75.0 ± 7.2 (150)^a,c,e,f</td>
<td>78.4 ± 8.2 (141)^f</td>
<td>80.2 ± 10.3 (129)^b</td>
<td>81.7 ± 11.3 (139)^g</td>
</tr>
<tr>
<td>BMI (kg.m^−2)</td>
<td>23.0 ± 1.5 (138)^a,b,h</td>
<td>24.1 ± 1.9 (129)^b</td>
<td>24.6 ± 2.5 (143)^f</td>
<td>25.0 ± 3.3 (139)^g</td>
</tr>
<tr>
<td>Overall Time (min)</td>
<td>655 ± 42 (152)^b</td>
<td>751 ± 26 (153)^b</td>
<td>860 ± 46 (133)^c.i</td>
<td>N/A</td>
</tr>
<tr>
<td>South African-born (%)</td>
<td>48.3 (71)^e,r,h</td>
<td>73.8 (110)^b,h,i</td>
<td>64.1 (98)^e,c,i</td>
<td>83.8 (119)^h,b,c</td>
</tr>
</tbody>
</table>

Except for the percentage South African-born, values are expressed as mean ± SD. The number of subjects (n) is in parentheses.

Fast Triath vs. Con: ^aP < 0.001,  ^bP < 0.005,  ^cP < 0.013,  ^dP = 0.005,  ^eP < 0.001,  ^fP < 0.009,  ^gP = 0.009,  ^hP < 0.001.

Mid Triath vs. Con:  ^iP = 0.006,  ^jP < 0.005,  ^kP < 0.001.

Slow Triath vs. Con:  ^lP < 0.001.

Fast Triath vs. Slow Triath:  ^mP < 0.001,  ^nP < 0.001,  ^oP = 0.091.
ACTN3 R577X polymorphism genotype (A) and allele (B) frequencies within the fastest (Fast Triath), middle of the field (Mid Triath) and slowest (Slow Triath) consenting Caucasian male finishers of the 2000 and/or 2001 South African Ironman triathlons, as well as the Caucasian male controls (Con).

There were no significant differences in either the genotype or the allele distributions between the three triathlete groups and the control group. The R577X genotype frequencies found in the control population were similar to those reported for an Australian Caucasian population (RR: 30%; RX: 52%; RX: 18%) (Yang et al. 2003), but different to those reported for a Finnish Caucasian population (RR: 45.0%; RX: 45.8%; RX: 9.2%) (Niemi & Majamaa, 2005). There is a large variation of reported R577X genotype frequencies within endurance athletes (Yang et al. 2003; Lucia et al. 2006; Niemi & Majamaa, 2005). However, the frequency of the 577XX genotype in the fastest triathletes included in this study (RR: 35.5%; RX: 47.4%; RX: 17.1%) was similar to that reported for Olympic level endurance runners (RR: 25.0%; RX: 57.7%; RX: 17.3%) (Lucia et al. 2006), and the distribution of all the R577X genotypes was comparable to that reported by Yang et al. (2003) for specialist endurance athletes of mixed sporting disciplines (RR: 31%; RX: 45%; RX: 24%). Although the R577X genotypes within ACTN3 were not associated with endurance performance in this population, the association of other polymorphisms within the ACTN3 gene cannot be excluded. It is however interesting to note that previous research in this population of triathletes has confirmed the association of other polymorphisms within genes encoding the angiotensin converting enzyme, the bradykinin β2 receptor, and nitric oxide synthase 3, with endurance performance (Collins et al. 2004; Saunders et al. 2006). For this reason, it is unlikely that the lack of association between ACTN3 and performance is due to methodological flaws in this study design.

The endurance phenotype is multi-factorial and no single physiological variable has been found to accurately predict endurance performance. Hence, in this study actual performance during the 226 km triathlon was used to define the phenotype. The differences in weight, BMI and age seen between the triathlete groups and controls are to be expected. The faster triathletes are younger and weigh less than the other triathlete groups. However, the ACTN3 genotype was not associated with any of these physiological or performance variables. In addition, the linear trend for the percentage of South African born individuals in the

<table>
<thead>
<tr>
<th>ACTN3 Genotype</th>
<th>RR</th>
<th>XX</th>
<th>RX</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>33.4 ± 8.9 (200)</td>
<td>32.7 ± 8.7 (120)</td>
<td>32.8 ± 8.8 (278)</td>
<td>0.715</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>180.6 ± 6.8 (171)</td>
<td>180.3 ± 7.2 (113)</td>
<td>180.8 ± 7.2 (259)</td>
<td>0.787</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>78.8 ± 9.5 (200)</td>
<td>79.0 ± 10.2 (121)</td>
<td>78.7 ± 9.6 (274)</td>
<td>0.951</td>
</tr>
<tr>
<td>BMI (kg·m⁻²)</td>
<td>24.3 ± 2.7 (180)</td>
<td>24.3 ± 2.5 (113)</td>
<td>24.1 ± 2.4 (256)</td>
<td>0.460</td>
</tr>
<tr>
<td>Overall Time (min)</td>
<td>752 ± 95 (162)</td>
<td>767 ± 98 (91)</td>
<td>753 ± 88 (204)</td>
<td>0.411</td>
</tr>
<tr>
<td>Swim Time (min)</td>
<td>70 ± 13 (156)</td>
<td>72 ± 15 (88)</td>
<td>70 ± 12 (196)</td>
<td>0.305</td>
</tr>
<tr>
<td>Bike Time (min)</td>
<td>389 ± 41 (151)</td>
<td>395 ± 46 (83)</td>
<td>387 ± 40 (191)</td>
<td>0.317</td>
</tr>
<tr>
<td>Run Time (min)</td>
<td>282 ± 49 (152)</td>
<td>290 ± 48 (90)</td>
<td>284 ± 47 (195)</td>
<td>0.442</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD. The number of subjects (n) is in parentheses.
The ACTN3 gene and elite endurance performance

The ACTN3 gene encodes the protein alpha-actinin 3, which is involved in the regulation of muscle contraction. Mutations in the ACTN3 gene have been associated with athletic performance, particularly in endurance events.

In a study of South African Ironman triathletes, researchers analyzed the ACTN3 genotypes and association with performance. They found that the R577X polymorphism, which results in the 577RR genotype, was associated with better endurance performance during Ironman triathlons. This finding corroborates recent studies reporting no association of the R577X polymorphism with performance in sprinting or power-based events.

The study also found that the 577X allele and the 577XX genotype were associated with stronger performance in extended endurance events such as ultra-marathons and Ironman triathlons. However, similar results were obtained when only the South African born triathletes and controls were analyzed.

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Disclosures

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References


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