Strength, power, fiber types, and mRNA expression in trained men and women with different ACTN3 R577X genotypes

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1Department of Laboratory Medicine, Division of Clinical Physiology, Karolinska University Hospital, Huddinge, Stockholm; 2Department of Physiology and Pharmacology, Karolinska Institutet, Stockholm; and 3Swedish Winter Sports Research Centre, Department of Health Sciences, Mid Sweden University, Östersund, Sweden

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Norman B, Esbjörnsson M, Rundqvist H, Österlund T, von Walden F, Tesch PA. Strength, power, fiber types, and mRNA expression in trained men and women with different ACTN3 R577X genotypes. J Appl Physiol 106: 959–965, 2009. First published January 15, 2009; doi:10.1152/japplphysiol.91435.2008.—α-Actinin isoforms, α-actinin-2 and α-actinin-3, encoded by their respective genes ACTN2 and ACTN3. ACTN2 is expressed in all muscle fiber types, while only type II fibers, and particularly the type IIb fibers, express ACTN3. ACTN3 (R577X) polymorphism results in loss of α-actinin-3 and has been suggested to influence skeletal muscle function. The X allele is less common in elite sprint and power athletes than in the general population and has been suggested to be detrimental for performance requiring high power. The present study investigated the association of ACTN3 genotype with muscle power during 30-s Wingate cycling in 120 moderately to well-trained men and women and with knee extensor strength and fatigability in a subset of 21 men performing isokinetic exercise. Muscle biopsies were obtained from the vastus lateralis muscle to determine fiber-type composition and ACTN2 and ACTN3 mRNA levels. Peak and mean power and the torque-velocity relationship and fatigability output showed no difference across ACTN3 genotypes. Thus this study suggests that R577X polymorphism in ACTN3 is not associated with differences in power output, fatigability, or force-velocity characteristics in moderately trained individuals. However, repeated exercise bouts prompted an increase in peak torque in RR but not in XX genotypes, suggesting that ACTN3 genotype may moderate responsiveness to training. Our data further suggest that α-actinins do not play a significant role in determining muscle fiber-type composition. Finally, we show that ACTN2 expression is affected by the content of α-actinin-3, which implies that α-actinin-2 may compensate for the lack of α-actinin-3 and hence counteract the phenotypic consequences of the deficiency.

ACTN2; exercise; physical performance; Wingate test

SKELETAL MUSCLE α-actinins are actin-binding proteins that constitute the major structural component of the Z-line (3, 5). The two isoforms, α-actinin-2 and α-actinin-3, expressed in humans are encoded by their respective genes ACTN2 and ACTN3 (24, 27). While ACTN2 is expressed in all muscle fiber types, only type II fibers, and particularly the least oxidative type IIb fibers, express ACTN3 (28, 33). Apart from their structural and mechanical significance, α-actinins may also, through interactions with other proteins, have a regulatory role by coordinating the contraction process or a signaling function by binding to the glycolytic enzyme fructose-1,6-biphosphatase (reviewed in Ref. 20).

A common nonsense mutation (R577X) in the ACTN3, resulting in a premature stop codon and lack of detectable protein in homozygous individuals for the ACTN3 null allele (XX genotype), has been demonstrated in the general human population with ~18% prevalence in Caucasians (28). This mutation seems not to compromise muscle function, suggesting that the other isoform, the α-actinin-2, can compensate for the lack of α-actinin-3 protein. Interestingly, the frequency of the mutated X allele, and especially of the fully α-actinin-3-deficient genotype (XX), is markedly lower in sprint and power athletes (26, 29, 30, 34). This may imply that the α-actinin-3 isoform is critical in any activity calling for extraordinary speed or power. In contrast, among endurance-trained athletes, the XX genotype is reported to be more common, indicating that lack of α-actinin-3 aids in athletic events requiring superior aerobic capacity (26, 29, 34). Similarly, loss of α-actinin-3 expression prompted a shift favoring aerobic metabolism in the knockout mouse model (6, 21, 22).

It has also been suggested that α-actinins play a role in muscle fiber-type differentiation through interactions with the Ca2+ - and calmodulin-dependent protein phosphatase calcineurin (20, 33). In line with this suggestion, a recent study reported that the type IIx fiber percentage was higher in RR than in XX genotype (33). While these findings contrast results from knockout mouse studies (21, 22), they may imply that ACTN3 expression might be associated with higher percentage of type II fibers, thus favoring sprint and power performance. In contrast, individuals with no ACTN3 expression may be predisposed to develop a higher percentage of type I fibers benefiting endurance performance.

It may also be that R577X mutation impacts responsiveness to strength and power training. For instance, individuals displaying the XX genotype show compromised isometric strength, yet greater gains following a resistance training regimen (9), while in a study by Delmonico et al. (11), individuals with the RR genotype show greater increases in knee extensor power following strength training than those with the XX genotype. The differences in elite performance and training response across ACTN3 genotypes may indicate that although the two skeletal muscle specific α-actinin isoforms may play similar roles, there are specific functions possessed by the α-actinin-3 that are not compensated for by the α-actinin-2.

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In this study, muscle power was assessed in 120 trained men and women by means of an all-out short-term exercise task. In addition, muscle strength and fatigability were investigated in a subset of 21 men with either RR or XX genotype. Furthermore, the association between ACTN3 genotype and fiber-type composition was investigated, and the mRNA levels of ACTN3 and ACTN2 were quantified and related to genotype and to the fiber-type composition. We hypothesized that the XX genotype, relative to the RR genotype, would show inferior muscle power and strength. It was further hypothesized that fiber-type composition and genotype would be correlated such that the XX genotype would show less type II fiber preponderance than the RR genotype and that the ACTN2 expression would be inversely related to the ACTN3 expression.

METHODS

General Design

Subjects in the present study were selected from a larger group of healthy volunteers comprising college students from the greater Stockholm area (n = 380) in which blood samples had been collected previously and genotyped for the R577X mutation of the ACTN3 gene. Subjects were subsequently selected, based on their ACTN3 genotype, to obtain groups with different ACTN3 genotypes of comparable sample size. Volunteers with body mass index > 30 kg/m², training < 2 h/wk, or who were nicotine users were excluded. One-hundred twenty men and women with RR, RX, or XX genotype, performed an all-out maximal 30-s exercise task (i.e., the “Wingate test”) on a cycle ergometer (2). Data, collected from 75 of these subjects, on the effects of AMP deaminase deficiency on sprint performance have been reported elsewhere (14). Subjects with AMP deaminase deficiency did not participate in the present study. A subset of 21 men with either RR or XX genotype, performed an all-out maximal 30-s exercise task (Table 1) and a subset of 21 men, with either RR or XX genotype in which the knee extensor in vivo torque relationship and fatigability were examined (Table 2). Based on a questionnaire, reporting health and physical activity history, all volunteers were engaged in various leisure-time sports activities, e.g., resistance exercise, individual or team ball sports, cycling, running, calisthenics, or aerobics. The subjects selected for the study were moderately to well trained (>3–4 yr of training; ~2–10 h/wk), yet not competitive athletes. The percentage of body fat was estimated from skinfold measurements (triceps, biceps, subscapula, and suprailiacal region) (12) and used to calculate fat-free weight (FFW).

Genotyping

Genomic DNA was isolated from peripheral blood using the QIAamp DNA extraction kit (Qiagen). Genotyping for the identification of the R577X mutation in the ACTN3 gene was performed by an allelic discrimination assay with fluorogenic probes (Perkin-Elmer ABI Prisma 7700 Sequence Detection System; Applied Biosystems). The primer sequences were ACGATCAGTTCAGGCAAACCT (forward) and ACCCTGGATGCCCATGATG (reverse), and the TaqMan probe sequences were TCGCTCTCGGTACGC and CGCCTCTGATCACG (polymorphism underlined). The probes were labeled with fluorescent dyes VIC and FAM, respectively. To verify the genotyping results, selected samples were also analyzed using a different method (matrix-assisted laser desorption ionization time-of-flight mass spectrometry; MALDI-TOF) (18). The two genotyping methods showed identical results.

Experimental Protocols

Wingate test. Subjects performed a 30-s exercise on a mechanically braked cycle ergometer (Cardionics) at maximal propelling speed against a resistance of 7.5% of the subject’s body mass (2). Flywheel revolutions were counted with a sensor-microprocessor assembly. Average power output for 5-s periods was recorded during exercise. Each subject performed two bouts of exercise with 20 min rest between the bouts, which is sufficient for full recovery of power with this exercise regimen (16). Average power outputs during the two bouts were calculated for each subject and related to body mass. Peak power (PP), i.e., the highest power output elicited during the test as the average over a 5-s period, and mean power (MP), i.e., the average

### Table 1. Anthropometric characteristics, training status, and Wingate performance data in men and women with different ACTN3 R577X genotypes

<table>
<thead>
<tr>
<th>ACTN3 Genotype</th>
<th>577 RR</th>
<th>577 RX</th>
<th>577 XX</th>
<th>Statistics, P Value</th>
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<tr>
<td>Sex:</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Men (n = 21)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, yr</td>
<td>24±2</td>
<td>23±3</td>
<td>27±5</td>
<td>24±5</td>
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<tr>
<td>Weight, kg</td>
<td>79±11</td>
<td>66±9</td>
<td>81±10</td>
<td>64±8</td>
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<tr>
<td>BMI, kg/m²</td>
<td>24±3</td>
<td>23±3</td>
<td>24±2</td>
<td>23±2</td>
</tr>
<tr>
<td>BF, % (n = 98)</td>
<td>16±6</td>
<td>28±5</td>
<td>18±5</td>
<td>28±7</td>
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<tr>
<td>FFW, kg (n = 98)</td>
<td>66±7</td>
<td>48±6</td>
<td>67±10</td>
<td>46±5</td>
</tr>
<tr>
<td>Average training, h/wk</td>
<td>5.6±2</td>
<td>5.6±3</td>
<td>5.0±2</td>
<td>4.5±3</td>
</tr>
<tr>
<td>PP, W/kg body wt</td>
<td>11.3±1</td>
<td>9.5±0.7</td>
<td>11.3±1</td>
<td>9.6±1</td>
</tr>
<tr>
<td>MP, W/kg body wt</td>
<td>10.1±1</td>
<td>8.6±0.7</td>
<td>10.3±0.6</td>
<td>8.7±0.9</td>
</tr>
<tr>
<td>FI-15, %</td>
<td>23±6</td>
<td>23±7</td>
<td>21±7</td>
<td>24±6</td>
</tr>
<tr>
<td>FI-30, %</td>
<td>46±6</td>
<td>42±5</td>
<td>45±9</td>
<td>43±6</td>
</tr>
</tbody>
</table>

Values are means ± SD. Statistical analysis of differences across the RR, RX, and XX genotypes refer to comparisons regardless of sex. BF: body fat; FFW: fat-free weight; PP: peak power; MP: mean power. Fatigue index (FI), i.e., the power decrease during the test, was calculated as a difference between PP and power at 15 s as a percentage of PP (FI-15); and as a difference between PP and power at 30 s as a percentage of PP (FI-30).
Table 2. Anthropometric characteristics, training status, and strength and fatigue data in men with different ACTN3 R577X genotypes

<table>
<thead>
<tr>
<th>ACTN3 Genotype</th>
<th>577 RR (n = 12)</th>
<th>577 XX (n = 9)</th>
</tr>
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<tbody>
<tr>
<td>Age, yr</td>
<td>25±2</td>
<td>26±5</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>85±12</td>
<td>74±7</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>25±3</td>
<td>23±2</td>
</tr>
<tr>
<td>BF, %</td>
<td>16±7</td>
<td>13±4</td>
</tr>
<tr>
<td>FFW, kg</td>
<td>71±6</td>
<td>65±5</td>
</tr>
<tr>
<td>Training, h/wk</td>
<td>4.3±3</td>
<td>3.1±2</td>
</tr>
<tr>
<td>MVC, N·m</td>
<td>329±79</td>
<td>302±41</td>
</tr>
<tr>
<td>Relative MVC, N·m/kg body wt</td>
<td>3.86±0.7</td>
<td>4.08±0.6</td>
</tr>
<tr>
<td>PT decrease 1–15, %</td>
<td>19±7</td>
<td>19±6</td>
</tr>
<tr>
<td>PT decrease 1–30, %</td>
<td>62±7</td>
<td>63±8</td>
</tr>
</tbody>
</table>

Values are means ± SD. MVC: maximal voluntary contractions; PT: peak torque. *P < 0.05, RR significantly different from XX. PT decrease is the decrease in torque during 30 maximal consecutive knee extensions. PT decrease 1–15 and PT decrease 1–30 are calculated as mean PT at repetition 15–16 and at repetition 28–29, respectively, both as percentage of mean PT at repetition 2–3.

Isokinetic exercise. Twenty one men (12 RR and 9 XX) performed maximal voluntary knee extensions (KE) using an isokinetic dynamometer (Cybex II, Lumex Inc, Ronkonkoma, NY) as described in detail elsewhere (17). To assess the in vivo torque-velocity relationship, maximal muscle actions were performed at 30, 60, 90, 120, 180, and 300°/s from 90 to 180° knee angle. The dynamometer moment arm was then positioned at 110° to allow subjects to perform and maintain maximal isometric actions for 4–6 s to determine maximal voluntary contraction (MVC). For any action or speed mode, two trials were allowed, and if peak force or torque differed more than 5%, additional trials were performed. Muscle fatigue was measured using protocol consisting of 30 maximal consecutive concentric KE, from 90° to full knee extension at 120°/s. Mean peak torque of repetitions 2–3, 15–16, and 28–29, respectively, was calculated and the resulting torque decline subsequently computed (31). Torque was measured using strain-gauge technique and recorded along with knee joint angle, measured by means of electrogoniometry, and the angular velocity preset with the dynamometer, using a Windows-based data-acquisition system (MuscleLab, Ergotest, AS, Langesund, Norway). Tests were performed on two occasions, 1 wk apart. The individual values used in the statistical analysis represent mean of values resulting from the two tests.

Fiber-Type Composition

In muscle biopsies from 63 individuals (XX, n = 23; RX, n = 17; RR, n = 23), fiber-type composition was determined using the myofibrillar ATPase histochemical stain at different preincubation pH allowing for identification of type I, IIa, IIb, and IIc fibers (13). Tissue sections were evaluated using Leica system BX60; digital camera Sony CDK-500 (Tokyo). At least 250 but no more than 700 fibers were counted per section. Myosin heavy chains characteristics, allowing for the denomination of type IIX or IId fibers, the equivalent of the type IIb fibers as assessed by means of the ATPase stain, were not determined.

mRNA Expression

Muscle samples for the extraction of total RNA were available from 32 individuals (XX, n = 21; RR, n = 11). Total RNA was extracted from tissue samples by the acid phenol method (8). Two micrograms of the total RNA was used for cDNA synthesis using Superscript reverse transcriptase (Life Technologies, Stockholm, Sweden) and random hexamer primers (Roche Diagnostics, Mannheim, Germany) in a total volume of 20 µl. Detection of the ACTN3 and ACTN2 mRNA was performed on an ABI-PRISM 7700 Sequence detector (Applied Biosystems, Foster City, CA). Oligonucleotide primers and TaqMan probes were purchased as Assays-on-Demand from Applied Biosystems (assay IDs: Hs00153812_ml (ACTN3) and Hs01152477_ml (ACTN2)). β-Actin was used as an endogenous (housekeeping) reference gene to correct for potential variation in cDNA loading and quantity, and the primers and TaqMan probe for detection of β-actin mRNA were purchased from Applied Biosystems (assay ID: 4310881E). All reactions were performed in duplicates, in 96-well MicroAmp optical plates, with a volume of 25 µl, and the analyses were performed according to the manufacturer’s instructions. Data were analyzed using the comparative Ct-method applying the formula 2−(Ct[housekeeping gene]−Ct[target gene]). The mRNA levels are reported in arbitrary units (AU).

Statistics

Factorial ANOVA was performed with ACTN3 genotype and sex as independent variables, and age, height, body mass, body mass index, percent body fat, lean body mass, and training index as dependent variables to compare these background variables of the different genotype groups. Factorial ANOVA or ANCOVA, with ACTN3 genotype as independent variable and the different measures of performance, fiber-type composition, and mRNA expression levels as dependent variables, were also employed to evaluate potential differences between the ACTN3 genotypes. Correlation between variables was tested by linear regression analysis. Statistical significance was accepted at P < 0.05.

RESULTS

Prevalence of R577X Polymorphism

Subjects in the present study were selected based on their ACTN3 genotype to obtain groups with different ACTN3 genotypes of comparable sample size. Thus the frequency of the X allele in the studied cohorts is not intended to reflect its prevalence in the general population and deviates from the Hardy-Weinberg equilibrium. The prevalence of the different ACTN3 genotypes analyzed in blood samples collected at random from 380 healthy individuals was RR = 31%, RX = 50%, and XX = 19%. This corresponds to R and X allele frequencies of 56 and 44%, respectively, and accords with previous reports on Caucasian populations (25, 34) of similar sample size and is in Hardy-Weinberg equilibrium.

Muscle Power

There were no significant differences observed in age, physical characteristics, or activity level across different ACTN3 genotypes (Table 1). Likewise, peak and mean power and fatigability output in the 30-s Wingate test were very similar.
for the different ACTN3 genotypes (Table 1, Fig. 1). Given the general sex-related differences in power output, we choose to report group data for men and women. However, no significant sex differences in the effect of ACTN3 genotype were observed (ANOVA interaction term, sex × genotype; P > 0.05). Hence statistical analysis of the result was performed on men and women combined.

Muscle Strength

RR genotypes showed greater body mass and FFW (Table 2). However, the two genotypes displayed no differences in MVC expressed in absolute terms or when related to body mass. The torque-velocity relationship profiles were very similar for the two ACTN3 genotypes (Fig. 2), and no differences (P > 0.05) in normalized peak torque between RR and XX were found at any measured angular velocity. Likewise, fatigability (i.e., mean peak torque decline over 30 actions) showed no difference across RR and XX (Table 2). Peak torque at all angular velocities measured (Fig. 3) showed increases in the second test, and this response tended to be different for RR (4–10%) and XX (−0.5–2%); (repeated-measures ANOVA: P = 0.055).

Fiber-Type Composition

The proportion of type I fibers was (mean ± SD) 55 ± 12, 57 ± 12, and 54 ± 17; of type IIa was 32 ± 12, 34 ± 10 and 35 ± 14; and of type IIb was 12 ± 10, 8 ± 5, and 11 ± 12 in XX, RX, and RR, respectively. There were no differences in fiber-type composition across the three genotypes. Fiber-type composition data across the three genotypes is presented separately for men and women (Table 3). Women showed higher proportion of type I (P = 0.014) and lower proportion of type IIb (P = 0.026) fibers than men. There was also a positive correlation (r = 0.377; P = 0.0005) between the proportion of type I fibers and training volume expressed as hours of training per week. Multiple regression analysis showed that training status (P = 0.011) and sex (P = 0.001) but not ACTN3 genotype (P = 0.816) had a significant effect on the proportion of type I fibers. Type IIb fiber percentage was significantly impacted by sex (P = 0.024), with no contribution to the effect of training index (P = 0.166) and genotype (P = 0.712).

mRNA Expression

The mRNA levels are reported in arbitrary units (AU) corrected to β-actin (Fig. 4). The ACTN3 mRNA level was (mean ± SD) 0.40 ± 0.4 AU in XX and more than 10-fold higher in RR (6.98 ± 5.5 AU; P < 0.001). Analyses of muscle samples from 11 individuals with RR genotype revealed a negative correlation between ACTN3 expression and %type I fibers (r = 0.693; P = 0.018), and a tendency toward a positive correlation between ACTN3 expression and %type IIb fibers.
The ACTN2 mRNA level was markedly higher than that of ACTN3, and there was no difference in ACTN2 expression between XX (296 ± 182) and RR (246 ± 136). However, when %type IIb fibers was used as a covariate in the statistical analysis, the expression of ACTN2 was higher \( (P = 0.037) \) in XX compared with RR.

**DISCUSSION**

ACTN3 R577X polymorphism results in loss of the \( \alpha \)-actinin-3 protein in individuals with the XX genotype \((28) \). Previous studies have reported that the frequency of this genotype exceeds that of the general population in endurance athletes, while being rare or nonexisting in sprint or power athletes \((26, 29, 30, 34) \). Thus the XX genotype appears to favor endurance and compromise power or sprint performance. Furthermore, the consequences of \( \alpha \)-actinin-3 deficiency in nonathletes are poorly understood, and collectively, studies of various subject groups are at odds \((9, 11, 19, 23, 33) \). Thus the present study examined the impact of ACTN3 genotype on muscle power in 120 trained, yet not competitive men and women, using an all-out 30-s exercise task. However, we failed to detect any differences in performance across the three ACTN3 genotypes. Hence, in this cohort of regularly physically active (including both endurance and resistance trained) subjects, it appears that the ACTN3 genotype is not an important determinant of muscle power or sprint performance. Previously \((14) \) we reported a marked difference \((-10\%) \) in muscle power across different AMPD1 genotypes, clearly justifying that this particular exercise challenge should be sensitive enough to reveal potential genotype-dependent differences.

We also conducted a comprehensive test battery to assess the in vivo knee extensor torque-velocity relationship and fatigability in a limited subject sample. Although these results should be treated with caution, it turned out that performance indexes measured were independent of ACTN3 genotype. The finding of no ACTN3 genotype influence on the torque-velocity relationship concords with a recent study by McCauley and coworkers \((23) \), yet contrasts the report of greater “high-speed” torque in the RR compared with the XX genotype showed by Vincent et al. \((33) \). It is noteworthy however, that the RR genotype in this latter study \((33) \) also possessed greater type IIx fiber preponderance than the XX genotype, and thus the cause-effect relationship in that study remains unclear. The present study measured muscle strength on two occasions 1 wk apart. Interestingly, while the RR genotype showed increased peak torque during the second test, the XX genotype showed no such response \((Fig. 3) \). We can only speculate if this differential response may be due to a boosting effect induced by the novel exercise bout in the RR genotype, and/or, given that type II fiber sarcomeres appear to be more vulnerable to Z-line disruption without the stabilizing influence of the \( \alpha \)-actinin-3 protein \((10, 15) \), if it merely reflects a compromised function and recovery from the previous intense exercise task in the XX genotype.

Previous studies indicate that ACTN3 genotype may impact adaptations to training in nonathletes reporting both greater \((9) \) and smaller \((11) \) gains in performance associated with XX genotype. It is also worth noting that the effect of ACTN3 genotype on performance and response to training appears to be greater in women than in men \((9, 11, 19, 32) \). Thus multiple factors, e.g., state of training, exercise mode, intensity, and

### Table 3. Muscle fiber-type composition in men and women with different ACTN3 R577X genotypes

<table>
<thead>
<tr>
<th>ACTN3 Genotype</th>
<th>577 RR</th>
<th>577 RX</th>
<th>577 XX</th>
<th>Statistics, ( P ) Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex:</td>
<td>Men (( n = 16 ))</td>
<td>Women (( n = 7 ))</td>
<td>Men (( n = 8 ))</td>
<td>Women (( n = 8 ))</td>
</tr>
<tr>
<td>Type I, %</td>
<td>49 ± 17</td>
<td>66 ± 7</td>
<td>54 ± 10</td>
<td>62 ± 13</td>
</tr>
<tr>
<td>Type IIa, %</td>
<td>39 ± 14</td>
<td>25 ± 8</td>
<td>36 ± 6</td>
<td>31 ± 13</td>
</tr>
<tr>
<td>Type IIb, %</td>
<td>12 ± 13</td>
<td>8 ± 5</td>
<td>9 ± 5</td>
<td>7 ± 6</td>
</tr>
</tbody>
</table>

Values are means ± SD. Statistical analysis of differences across the RR, RX, and XX genotypes refer to the comparison regardless of sex.

\( r = 0.507; P = 0.112 \). Fig. 4. ACTN3 and ACTN2 mRNA levels in men and women with different ACTN3 R577X genotypes. Values are expressed in arbitrary units (AU) corrected to \( \beta \)-actin and shown as means and SEs. The ACTN3 mRNA level was significantly higher in RR than in XX: \( P < 0.001 \).
frequency, muscle fiber-type composition; and sex of the examined subject samples, may account for some of the contrasting findings reported. Certainly, additional studies are requested to elucidate the impact of ACTN3 genotype on the response to specific acute and chronic exercise challenges.

Nonetheless, recent studies (1, 26, 29, 30, 34) do suggest that XX genotype, in part, is of disadvantage for performance in athletic events calling for high speed and power. Our results are not at odds, and the observed greater increase in peak torque in RR than in XX (Fig. 3) may imply that the ACTN3 genotype could modulate the response to training and induce differences in performance between XX and RR genotype when specific exercise bouts are chronically imposed.

It has been put forth that α-actinin-3 influences fiber-type differentiation by promoting glycolytic type II fibers, while lack of α-actinin-3 would lead to a higher proportion of oxidative type I fibers (20). In support, the RR genotype showed higher percentage of type IIx fibers than the XX genotype (33) in a group of men where resistance-trained subjects were excluded. The present study examining men and women engaged in a large variety of sports activities found no differences in fiber types across the three genotypes but rather that the training status and sex, not ACTN3 genotype, had a significant effect on the fiber-type composition. Studies employing the knockout mouse model suggest that adaptation of the α-actinin deficient muscle occurs via signaling pathways involved in the regulation of both size and metabolic properties of muscle fibers, rather than their myosin heavy chain composition (21, 22).

Lack of α-actinin-3 in the XX genotype seems not to compromise muscle function, suggesting that the other isoform, the α-actinin-2, may compensate for the lack of α-actinin-3 protein. The present study aimed at exploring the interaction between the two isoforms and determining the extent of compensation for the lack of ACTN3 expression by expression of ACTN2. The mRNA levels of ACTN3 and ACTN2 were quantified and related to ACTN3 genotype and fiber-type composition. The observed strong negative correlation between ACTN3 mRNA expression and the percentage of type I fibers in the RR genotype is commensurate with ACTN3 expression being confined to type II fibers (24, 28, 33). Furthermore, the lack of association between the ACTN2 expression and fiber-type composition conforms with previous notions that all muscle fiber types express α-actinin-2 (24, 33). In addition, our results demonstrate that the expression of ACTN2 is affected by the content of α-actinin-3, since ACTN2 expression was significantly higher in XX than in RR when the percentage of type IIb fibers was used as a covariate. These findings strongly support that the α-actinin-2 compensates for the lack of α-actinin-3.

We also showed a robust downregulation of ACTN3 mRNA levels in the XX genotype, indicating that skeletal muscle is protected from translation of the truncated α-actinin-3 protein. The α-actinins are composed of two antiparallel subunits assembled into homodimers (5). The two α-actinin isoforms are coexpressed in type II fibers and may be assembled into α-actinin-3/2 heterodimers (7). Downregulation of the ACTN3 mRNA expression protects skeletal muscle from the potentially harmful effects of the defective α-actinin-3 protein and may eliminate formation of dysfunctional heterodimers with one defective subunit with less need for compensatory upregulation of the ACTN2. A previous study found no differences in the amount of the α-actinin-2 protein between XX and RR (33). The much higher expression of ACTN2 than ACTN3 at the mRNA level in RR genotype found in the present study may imply that also at the protein level the α-actinin-2 expression is more substantial than the expression of α-actinin-3. Hence, even small increases in the α-actinin-2 content may be sufficient to compensate for deficiency of α-actinin-3. Such small differences in the content of α-actinin-2 may be more difficult to detect with use of the immunohistochemical method.

In summary, the present study demonstrates that R577X polymorphism in ACTN3 is not associated with differences in power output, fatigability, or force-velocity characteristics in physically active individuals. Our results, however, do not exclude that ACTN3 genotype may modulate responsiveness to training and as a consequence induce differences in athletic performance. The present data further suggest that α-actinin-2 do not play a significant role in determining muscle fiber-type composition. Finally, we show that the expression of ACTN2 is affected by the content of α-actinin-3, which implies that α-actinin-2 may compensate for the lack of α-actinin-3 and hence counteract the phenotypic consequences of the deficiency.

ACKNOWLEDGMENTS

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GRANTS

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