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ACTN3 (R577X) genotype is associated with fiber type distribution

Barbara Vincent, Katrien De Bock, Monique Ramaekers, Els Van den Eede, Marc Van Leemputte, Peter Hespel, and Martine A. Thomis

Research Centre for Exercise and Health, Department of Biomedical Kinesiology, Faculty of Kinesiology and Rehabilitation Sciences, Katholieke Universiteit Leuven, Leuven, Belgium

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Vincent B, De Bock K, Ramaekers M, Van den Eede E, Van Leemputte M, Hespel P, Thomis MA. ACTN3 (R577X) genotype is associated with fiber type distribution. Physiol Genomics 32: 58–63, 2007. First published September 11, 2007; doi:10.1152/physiolgenomics.00173.2007.—α-Acnitin-3 is a Z-disc structural protein found only in type II muscle fibers. The X allele of the R577X polymorphism in the ACTN3 gene results in a premature stop codon and α-actinin-3 deficiency in XX homozygotes. Associations between the R577X polymorphism and the muscle-power performance of elite athletes have been described earlier. About 45% of the fiber type proportions are determined by genetic factors. The ACTN3 variant could be one of the contributing genes in the heritability of fiber type distribution through its interaction with calcineurin. The aim of this study was to quantify the association between the polymorphism and muscle fiber type distribution and fast-velocity knee extension strength. Ninety healthy young men (18–29 y) were genotyped for ACTN3 R577X. Knee extension strength was measured isometrically (45°) and at different dynamic velocities (100–300°/s) on a programmable dynamometer. Twenty-two XX and twenty-two RR subjects underwent a biopsy of the right vastus lateralis muscle. Fiber type composition was determined by immunohistochemistry. Homozygotes for the R allele show significantly higher relative dynamic quadriceps torques at 300°/s, compared with XX carriers (P < 0.05). Fiber type characteristics differed significantly between the two genotype groups. The percentage surface and number of type IIx fibers were greater in the RR than the XX genotype group (P < 0.05), and α-actinin-3 protein content is systematically higher in type IIx compared with type IIA fibers (staining intensity ratio IIx to IIA = 1.17). This study shows that the mechanism, by which the ACTN3 polymorphism has its effect on muscle power, might rely on a control function of fiber type proportions.

α-actinin; muscle genetics; immunohistochemistry; fiber typing

The ACTN3 gene encodes the protein α-actinin-3. α-Actinin-3 is an actin-binding protein that is structurally related to dystrophin (6). In humans, two genes encode for skeletal muscle α-actinins: ACTN2, which is expressed in all skeletal muscle fibers, vs. ACTN3, whose expression is limited to fast-twitch muscle fibers (100% of type IIdx fibers and 50% of type Ila fibers; Refs. 16, 17). α-Actinins are important structural components of the Z-membrane (1) where they form the crosslink between the thin actin filaments. They have a static function in maintaining ordered myofibrillar arrays and a regulatory function in coordinating myofiber contraction (13). Interestingly, in European Caucasian populations ~18% of the individuals are fully α-actinin-3 protein deficient due to homozygosity for a premature stopcodon polymorphism in the ACTN3 gene (Chrom 11, pos. 66084671, C→T, R577X, rs1815739). However, this deficiency does not result in a disease phenotype or muscular functional impairment (10, 11, 17). Still, a number of studies have provided data to indicate that there is a positive association between the presence of the R allele and the capacity to perform high power muscle contractions (14, 15, 24). On the other hand, the X allele might predispose for better endurance exercise performance (14, 15, 24). Accordingly, Yang et al. (24) found in a sample of white elite athletes a higher frequency of the 577R allele in both male and female sprinters, while elite endurance athletes exhibited a slightly higher frequency of the XX genotype.

α-Actinins interact with themselves, structural proteins of the contractile machinery, metabolic enzymes, and signaling proteins (reviewed in Ref. 10, among them are also members of the Z-line localized calcinar family (8). These bind to calcineurin, a Ca2⁺ and calmodulin-dependent protein phosphatase, which is a signaling protein and is hypothesized to play a role in the determination of muscle fiber type and muscle hypertrophy (10), although it does not seem to be implicated in muscle fiber growth in regenerating muscle (19). Semsarian et al. (20) showed that in reaction to intracellular calcium mobilization, calcineurin is activated. The latter in turn causes a nuclear translocation of the transcription factor nFATc1. In rats, the activation of calcineurin mobilizes satellite cells and causes a switch to a more glycolytic metabolism (20). On the contrary, Chin et al. (2) reported that the activation of calcineurin selectively up-regulates slow-fiber-specific gene promoters.

Based on genetic epidemiological studies, about half of the variability in fiber type distribution in human muscles is determined by genetic factors (21). Through its interaction with calcineurin, polymorphisms in the ACTN3 gene could conceivably contribute to heritability of fiber type distribution. The force-generating capacity of type II muscle fibers at high velocity, the speed of movements, and the capacity to adapt to training are all strongly genetically influenced (24). The contribution of genetic factors in strength measures in part varies according to the angle, to the contraction type, and to some extent the contraction velocity (23). Contractile property differences according to the presence/absence of α-actinin-3 in sarcomeres of fast-type muscle fibers might also contribute to individual differences in power output.

Currently, not much is known about the effect of the α-actinin-2/3 protein content in the muscle. North and Beggs (16) observed in 1996 that the α-actinin-2/3 protein content is fiber type dependent. They show that while α-actinin-2 is found in all skeletal fibers, α-actinin-3 is present in only a subset of type II fibers (all type IId fibers and 50% of the type Ila fibers), although no numeric information was reported previously (16).
This issue raises the question whether the content of these proteins differs between individuals, and if so, whether this difference can explain variation in performance.

The primary purpose of this study was to investigate the relationship between the ACTN3 (R577X) genotype and muscle fiber type distribution in humans on the one hand and the capacity of force generation of the muscle fibers (at different velocities) on the other hand. The secondary aim was to investigate the relationship between fiber type specific α-actinin-2 and -3 protein levels and skeletal muscle performance.

Because of the exclusive prevalence of α-actinin-3 in fast glycolytic (type II) muscle fibers and the interaction between the α-actinins and calcineurin (with its likely function in fiber type determination), we proposed the following hypotheses. 1) Subjects with α-actinin-3 deficiency have lower baseline muscle power than subjects with α-actinin-3, and this difference in dynamic strength becomes more obvious with increased velocity of contraction; 2) ACTN3 is essential for the differentiation and structural traits of muscle fibers and explains part of the inter-individual differences in fiber type distribution; and 3) the observed differences in power output can at least in part be explained by a difference in α-actinin-2/3 protein content.

MATERIALS AND METHODS

Subjects

Ninety healthy young males (age: 21.7 ± 2.3 y; body wt: 73.3 ± 8.6 kg) gave written consent to participate after being fully informed of the study protocol and procedures. The Ethics Committee of the Faculty of Medicine of Katholieke Universiteit Leuven approved the study protocol. All experiments were conducted in conformity with the principles of the declaration of Helsinki. The subjects were recruited by announcements among the local student population. Inclusion criteria on admission were male, ages 18–30 y, and in good health. Exclusion criteria were acute or chronic disease, consistent intake of medication or nutrition supplements of any kind during a period of 6 mo before the study, any medical condition that might contra-indicate high-intensity exercise, and a prehistory of consistent resistance training in a period of 12 mo before the study.

Study Protocol

The study was performed in two phases. In phase I, the relationship between the ACTN3 (R577X) polymorphism and muscle strength was studied in the total group (n = 90) of subjects. In phase II, a subgroup was used to compare muscle fiber type distribution and muscle strength between RR (n = 22) and XX (n = 22) ACTN3 homozygotes. Furthermore, in the latter subgroup muscle α-actinin-2/3 protein content was also determined.

In phase I, subjects reported twice to the research center within a period of 6 mo before the study, any medical condition that might contra-indicate high-intensity exercise, and a prehistory of consistent resistance training in a period of 12 mo before the study.

Analyses of Blood and Muscle Samples

SNP genotyping. DNA was extracted using the Chemagic DNA Blood Kit on an automated Chemagic Magnetic Separation Module I (Chemagen, Baesweiler, Germany) and a Multiprobe I (PerkinElmer, Waltham, MA) robotic station.

Genotyping was performed using a TaqMan SNP genotyping assay (Applied Biosystems), containing a 20× mix of unlabeled PCR forward and reverse primers as well as a VIC and FAM labeled allele mix, and 10× of the 2× Taqman universal PCR master mix (Applied Biosystems). Amplification and detection were performed using the ABI PRISM 7300 sequence detection system (Applied Biosystems). Thermal cycling conditions were 10 min at 95°C followed by 40 two-step cycles, including 15s denaturation at 92°C and 60 s annealing/extension at 60°C. All reactions were set up manually, and allele calling was done using SDS 1.3 software.
**Muscle histochemistry.** Serial sections (4 μm) from biopsy samples were collected on uncoated glass slides. Briefly, cryosections were fixed for 10 min in 4% paraformaldehyde in PBS. Slides were rinsed for 2 × 5 min with wash buffer (0.5% BSA in PBS), treated with 10 mm NH4Cl, and washed again (2 × 5 min). Slides were prehybridized in 1% BSA in PBS for 30 min. Sections were then incubated overnight at 4°C with the primary antibodies. The incubation was followed by 3 × 5 min washes with wash buffer, after which the appropriate conjugated antibodies were added. Finally, the sections were washed again (3 × 5 min in wash buffer) and coverslips were mounted with fluorescent mounting medium (DakoCyton, Carpinteria, CA). For muscle fiber typing we used primary antibodies directed against human myosin heavy chain I and IIa (A4.840 and N2.261 supernatant from Developmental Studies Hybridoma Bank at the University of Iowa, Iowa City, IA). The primary antibodies against α-actinin-2 and α-actinin-3 were sera and affinity purified rabbit polyclonal antibodies raised against amino-terminal peptides, rabbit anti-α-actinin-2 rod raised against a peptide from the central rod domain, and monoclonal mouse anti-merosin M-chain (16). Fiber type specific stainings for α-actinin-2 and α-actinin-3 protein content were performed in separate experiments. Pilot experiments revealed no cross-reactions between different primary and secondary antibodies.

The secondary antibodies for fiber typing were FITC anti-mouse IgM (Southern Biotechnology Associates, Birmingham, AL) and Alexa Fluor 350 anti-mouse IgG1 (Molecular Probes, Leiden, the Netherlands) for type I and type IIa fibers, respectively, and anti-rabbit IgG (Abcam, Acris, Germany) for α-actinin-2/3. Slides were examined using a Nikon E1000 fluorescence microscope (Nikon, Boerhavedorp, Germany) equipped with a digital camera. Epifluorescence signal was recorded using a FITC, DAPI, and Texas red filter for type I muscle fibers, type IIa muscle fibers, and α-actinin-2/3 protein content, respectively, using standardized camera and microscope settings. Captured images (×20 magnification) were processed and analyzed using Lucia G software (LIM, Prague, Czech Republic). Fibers, negatively stained for type I and type IIa, were qualified as type IIx fibers. To eliminate inter-assay variation, samples aimed for mutual comparison were consistently included in the same assay. Background correction was performed by adding negative control samples in each assay.

Forty-three muscle samples were included in the fiber typing analysis. The number of fibers analyzed per sample was 170 ± 11. The intra-assay coefficient of variation for fiber type proportions and surface areas was 5%.

### Statistical Analysis

The statistical analyses were done with SAS 9.1 and Statistica 6 software. An ANOVA was performed to evaluate an association effect between the strength phenotypes and the three genotype groups to investigate a possible co-dominant effect. An estimation of the cross-sectional area of the quadriceps was used as a covariate in an ANCOVA analysis. To determine possible dominant allele effects, independent sample t-tests were performed (have X allele vs. have no X allele; have R allele vs. have no R allele). To test for a possible contraction-velocity interaction by different ACTN3 genotype groups, a repeated measures ANOVA was applied to raw and relative torque values at different contraction speeds.

Student’s t-tests were also used to determine differences between the two selected genotype groups (RR vs. XX, n = 43) in the fiber typing analyses. Pearson (or Spearman) correlations were used to study correlations between staining values for α-actinin-2/3 fiber-specific protein content and performance or fiber composition phenotypes. Multiple regression analysis was added to implement the cross sectional area of the quadriceps and fiber type proportions as covariate factors. A probability level (P) < 0.05 was considered statistically significant. All data are expressed as means ± SE.

### RESULTS

#### Prevalence of ACTN3 R577X Polymorphism and Body Composition

The proportion of XX, RX and RR genotypes in the study sample was 0.24, 0.44, and 0.31, respectively. These frequencies are in Hardy-Weinberg equilibrium (Chi² value = 0.95; P = 0.33). The allele frequencies are 0.47 and 0.53 for the X allele and R allele, respectively. Body composition characteristics as well as estimated quadriceps muscle cross-sectional area were similar between the three genotypes (Table 1).

#### Muscle Fiber Type Distribution

Muscle fiber type distribution was measured in biopsy samples obtained from m. vastus lateralis (Table 2). On average the relative fraction of type I, IIa, and IIx fibers was 52, 36, and 12%, respectively, in the total group. Genotype-specific differences were found for the percentage number of type IIa fibers, which was 5% higher in the RR than in XX genotype group (P = 0.04). Given similar average surface area per type IIx fiber, the relative muscle surface area covered by type IIx fibers was also slightly greater in the RR than in the XX genotype group (P = 0.03). There were no significant differences between the genotype groups for either type I or type IIa fiber number or surface area.

#### α-Actins-2/3 Protein Content

Muscle α-actin-2/3 protein content was determined using immunohistochemical assays (see Fig. 1). Staining intensity for α-actinin-3 protein in type II fibers on average was 80% higher in RR than in XX, which confirms the identification of the polymorphism (P < 0.001; data not shown). Staining intensity was similar between type I fibers in RR and any fiber type in XX, which indicates the lack of expression of α-actinin-3 protein in type I fibers (P = 0.72; data not shown). The expression of α-actinin-3 protein content was compared between type IIa and type IIx fibers in RR subjects exhibiting a sufficient number (>10) of type IIx fibers in the muscle sections (n = 11; Fig. 2). Staining intensity for α-actinin-3 protein content largely varied among the different subjects. However, compared with type IIa fibers, α-actinin-3 average red staining in type IIx fibers on average was ~17% higher (P = 0.04), and this difference was consistent in all but one individual. Average red staining for α-actinin-2 protein content was similar between the two genotype groups (data not shown).

### Table 1. Anthropometric characteristics by ACTN3 R577X genotype in total sample

<table>
<thead>
<tr>
<th></th>
<th>577XX (n = 22)</th>
<th>577RX (n = 40)</th>
<th>577RR (n = 28)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Height, cm</td>
<td>181 ± 1.0</td>
<td>181 ± 0.9</td>
<td>179 ± 1.1</td>
</tr>
<tr>
<td>Body weight, kg</td>
<td>74 ± 1.7</td>
<td>74 ± 1.6</td>
<td>72 ± 1.2</td>
</tr>
<tr>
<td>Body fat, %</td>
<td>12.9 ± 0.7</td>
<td>13.3 ± 0.6</td>
<td>12.8 ± 0.7</td>
</tr>
<tr>
<td>Fat mass, kg</td>
<td>9.6 ± 0.6</td>
<td>11.6 ± 1.9</td>
<td>9.2 ± 0.6</td>
</tr>
<tr>
<td>Fat free mass, kg</td>
<td>63.9 ± 1.2</td>
<td>62.6 ± 1.5</td>
<td>62.0 ± 1.0</td>
</tr>
<tr>
<td>Estimated m. quadriceps cross-sectional area, cm²</td>
<td>69.1 ± 2.1</td>
<td>70.4 ± 1.5</td>
<td>69.0 ± 1.8</td>
</tr>
</tbody>
</table>

Mean ± SE are given. Body composition data are from a subgroup of the total sample (n = 90) (XX: n = 19; RX: n = 40; RR: n = 26).
The ACTN3 (R577X) polymorphism causes a complete loss of the α-actinin-3 protein in XX homozygotes. About 18 percent of European-ancestry populations are ACTN3 deficient, with no obvious related pathology. The polymorphism has been associated with elite athletic performance; the R allele was more common in sprint and power athletes, while the X allele was more frequent in endurance athletes (10, 11, 14, 15, 24). Through an interaction with the signaling protein calcineurin, Yang et al. (24) proposed that α-actinin-3 might promote the formation of fast twitch fibers.

Here, we tested whether the R577X genotype is associated with baseline muscle strength (dynamic torque), although more specifically the association of this polymorphism with fiber type proportions and characteristics in healthy young men. We also documented fiber type specific α-actinin-2/3 protein contents. We took blood samples from 90 men to determine their R577X genotype. These subjects performed several strength measurements at different velocities on a computerised isokinetic dynamometer. From 44 of these subjects (22XX and 22RR carriers), a muscle biopsy was taken for the analysis of fiber type proportions and fiber type specific α-actinin-2/3 protein content using immunohistochemical assays.

The relative allele frequency of the 577X allele for our study population was 0.47. This is similar to the frequencies previously reported (3, 13, 14, 24). Our population frequency for XX homozygotes was 24%, which is slightly higher than the 18% found for Europeans by Yang et al. (24). This may be due to the specific characteristics of our research population, which consisted mainly of physically active young men (18–29 y).

We found no association between the ACTN3 genotype and anthropometric or body composition characteristics. These findings are similar to the study of Moran et al. (14).

XX homozygotes showed significantly less (relative) dynamic muscle power than homozygotes for the wild-type allele (total group and subgroup analysis). In the total group analysis, heterozygotes were intermediate between both homozygotes for most of the quadriceps torque values, indicating a co-dominant gene action. These findings expand on earlier studies that mostly used a case-control approach (15, 25) including elite athletes. Our findings show an additive effect of each R allele to enhance power in healthy nonathlete young men (18–29 y).

**DISCUSSION**

Muscle Strength

Muscle strength was measured as torque production during maximal static and dynamic knee extensions on an isokinetic dynamometer. As shown in Table 3 absolute torque production was similar among the three genotype groups for either static, dynamic concentric, or eccentric contractions. However, dynamic torque production expressed relative to maximal static torque was different among genotypes for the highest contraction speed. At 300°/s, the RR genotype group showed higher relative knee extension torques than the XX group (P = 0.04). As shown in Fig. 3 for the XX and RR groups, following the strength-velocity relationship, relative torque production decreased as contraction velocity increased. However, this decrease was greater in the XX than in the RR group (genotype by velocity interaction: P = 0.06). Similar results were found when estimated quadriceps cross-sectional area, body mass index, or weight was included as a covariate.

Within the RR genotype, there was no correlation between muscle α-actinin-3 staining intensity and torque output during high velocity (300°/sec) muscle contractions, even after correction for differences in fiber type distribution in multiple regression analysis.

**Table 2. Effect of ACTN3 R577X polymorphism on muscle fiber type composition**

<table>
<thead>
<tr>
<th>Fiber Type</th>
<th>577XX (n = 21)</th>
<th>577RR (n = 22)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type I</td>
<td>55±3</td>
<td>50±2</td>
</tr>
<tr>
<td>Type IIa</td>
<td>35±2</td>
<td>37±2</td>
</tr>
<tr>
<td>Type IIx</td>
<td>9±1</td>
<td>14±2*</td>
</tr>
</tbody>
</table>

Average surface area per fiber type, μm²

<table>
<thead>
<tr>
<th>Fiber Type</th>
<th>577XX (n = 21)</th>
<th>577RR (n = 22)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type I</td>
<td>4,265±165</td>
<td>4,404±204</td>
</tr>
<tr>
<td>Type IIa</td>
<td>5,318±243</td>
<td>5,611±330</td>
</tr>
<tr>
<td>Type IIx</td>
<td>4,581±272</td>
<td>5,095±349</td>
</tr>
</tbody>
</table>

Relative surface area per fiber type, %

<table>
<thead>
<tr>
<th>Fiber Type</th>
<th>577XX (n = 21)</th>
<th>577RR (n = 22)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type I</td>
<td>51±3</td>
<td>45±2</td>
</tr>
<tr>
<td>Type IIa</td>
<td>40±3</td>
<td>42±2</td>
</tr>
<tr>
<td>Type IIx</td>
<td>7±1</td>
<td>12±2*</td>
</tr>
</tbody>
</table>

Values are means ± SE of 43 observations. Muscle fiber typing was performed by immunohistochemistry on biopsy samples obtained from m. vastus lateralis. See materials and methods for further details. *P < 0.05 vs. 577XX genotype.

**Fig. 1.** Immunohistochemical determination of fiber type specific α-actinin-3 protein content. This figure shows the fiber type specific staining of α-actinin-3 in an RR-carrier subject. The bright blue fibers (A) correspond to type IIa fibers, which also contain the α-actinin-3 (bright red staining in B). There is no α-actinin-3 in the fibers staining green (type I fibers). The darker blue fibers (type IIx fibers) show relatively more α-actinin-3 protein content than the type IIa fibers in this subject.
Fig. 2. Effect of fiber type on α-actinin-3 protein content. Data are individual values expressed in arbitrary units corresponding to red staining intensity corrected for background staining. Open bars, type IIa fibers; filled bars, type IIx fibers. See MATERIALS AND METHODS for further details.

Table 3. Effect of ACTN3 polymorphism on muscle strength

<table>
<thead>
<tr>
<th></th>
<th>577XX (n = 22)</th>
<th>577RX (n = 40)</th>
<th>577RR (n = 28)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absolute torque</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Static torque</td>
<td>211 ± 6</td>
<td>207 ± 4</td>
<td>195 ± 5</td>
</tr>
<tr>
<td>Dynamic torque</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>100°/s</td>
<td>131 ± 6</td>
<td>137 ± 5</td>
<td>127 ± 6</td>
</tr>
<tr>
<td>200°/s</td>
<td>84 ± 6</td>
<td>89 ± 4</td>
<td>87 ± 5</td>
</tr>
<tr>
<td>300°/s</td>
<td>56 ± 6</td>
<td>59 ± 4</td>
<td>56 ± 5</td>
</tr>
<tr>
<td>Eccentric torque</td>
<td>219 ± 7</td>
<td>215 ± 3</td>
<td>212 ± 4</td>
</tr>
<tr>
<td>Relative torque</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>100°/s</td>
<td>0.62 ± 0.02</td>
<td>0.66 ± 0.02</td>
<td>0.65 ± 0.02</td>
</tr>
<tr>
<td>200°/s</td>
<td>0.40 ± 0.02</td>
<td>0.43 ± 0.01</td>
<td>0.45 ± 0.02</td>
</tr>
<tr>
<td>300°/s</td>
<td>0.26 ± 0.03</td>
<td>0.28 ± 0.02</td>
<td>0.34 ± 0.02*</td>
</tr>
</tbody>
</table>

Data are means ± SE of 90 observations. Absolute torques are expressed in Nm. See MATERIALS AND METHODS for further details. *P = 0.04 vs. 577XX genotype.

Fig. 3. Effect of ACTN3 R577X polymorphism on knee extension force-velocity curve. Data are means ± SE of 43 observations and are expressed as the ratio of dynamic torque over maximal static torque. ■, 577RX genotype; ●, 577RR genotype. Relative dynamic torque of the knee extensor muscle was measured on an isokinetic dynamometer at angular velocities of 100, 200, and 300°/s. *P = 0.05 vs. 577XX.

Other research groups were not able to detect a significant increased muscular strength/power in the untrained state (3, 4); however, location (elbow joint), sample ethnicity, and ages were different from the present study.

The major aim of our study was to investigate the role of the ACTN3 (R577X) polymorphism in the determination of fiber type characteristics. We hypothesized that the associations between the ACTN3 (R577X) polymorphism and fast-speed isokinetic isometric torque could be explained by increased type II muscle fiber differentiation (in RR carriers) and therefore the force delivering capacity of the type II muscle fibers in dynamic movements.

This role of ACTN3 could be through a binding of α-actinin-3 with calsarcins that interact with the signaling protein calcineurin to promote the formation of fast twitch fibers (24).

Calcineurin, a serine-threonine phosphatase activated by Ca2⁺-calmodulin, participates in signaling pathways important for gene regulation and biological responses to external stimuli in many organisms and in many types of cells (5, 18). Chin et al. (2) showed that a signaling pathway that involves calcineurin controls fiber type specific gene expression in skeletal muscles. They identified a molecular mechanism by which different patterns of motor nerve activity selectively promote changes in gene expression to establish the specialized characteristics of slow and fast myofibers (2). Semsarian et al. (20) concluded that calcineurin promotes a switch to a more glycolytic metabolism. In IGF-1 injected rats, activated calcineurin mobilized satellite cells and increased activity of glycolytic enzymes and the end product of the glycolytical metabolism (lactate) (20).

However, contrary findings are described in the study of Swoap et al. (22), where they show that active calcineurin is not sufficient to differentially regulate fiber type specific gene expression in whole muscle or in cell culture. Furthermore, Serrano et al. (19) state that calcineurin activity in muscle fibers is required for the induction and the maintenance of the slow muscle gene program and the repression of the fast MyHC-IIx genes. Contrary to the findings of Serrano et al., Michel et al. (12) conclude that calcineurin signaling primarily leads to an adaptation towards a more metabolically efficient phenotype in response to increased muscle usage. Hence, calcineurin is more than a signaling agent for exclusive maintenance of slow type I fiber profiles in response to ‘slow/chronic’ patterns of nerve activation (12). Dunn et al. (7) also present a more differentiated role for calcineurin than the sole maintenance of the slow gene program. They state that calcineurin signaling increases in all fiber types with increased nerve-mediated activity. Highly relevant to the findings in this study, most reactivity was found in the less active (IIx) fibers. The authors hypothesize that the transcription of proteins is only increased when activation is above the “native levels” threshold. Cells that already have a high calcineurin activity (type I fibers) become less sensitive for relatively small amounts of increased nerve activity (7).

In this study, we show a possible role of the ACTN3 gene in the determination of fiber type distribution. We found a positive association between the ACTN3 RR genotype and the amount and fiber surface of fast, glycolytic fibers (IIx) in terms of percentage (P < 0.05). These data are in agreement with the hypothesis of Yang et al. (24) that α-actinin-3 promotes the formation of fast-twitch fibers. These findings suggest that the mechanism, by which the ACTN3 (R577X) polymorphism has its effect on muscle power, might rely, at least in part, on the regulation of fiber type proportions. However, these findings do not exclude that other signaling pathways and interactions with metabolic enzymes also play a role in the α-actinin-3 specific effects on the regulation of muscle fiber type distribution in humans (10).

Finally, we hypothesized that the differences found in dynamic muscle torque might in part rely on a different amount of α-actinin-3 protein in the fast muscle fibers in the homozygous genotype.
gotes for the R allele. We were unable to confirm this hypothesis. We found no correlation between the amount of α-actinin-3 staining in the fast fibers and the muscle performance at high velocity. However, for the speed at 100 and 200°/s a trend towards a positive correlation was observed for both α-actinin-3 red staining in type IIa and type IIx fibers. Variability within the RR group for high-velocity contractions is probably related to many more genetic and nongenetic factors for which α-actinin-3 protein variability would be only one contributing factor. Probably the relatively small sample size of subjects in the RR group with α-actinin-3 protein level information and limited effect size of the α-actinin-3 protein level do not warrant definite conclusions.

North and Beggs (16) included unpublished observations concerning the partition of α-actinin-3 among type II fibers. They observed that the protein is present in all the type IIb fibers and 50% of the type IIa fibers. In our study, population of healthy young men, α-actinin-3 was present in all type IIa fibers of RR carriers, and we found systematic higher levels of α-actinin-3 in IIX fibers (staining intensity ratio of IIX to IIa: 1.17 ± 0.11). We are not aware of any other study reporting such data. For reasons of improved reliability, the analysis was restricted to RR individuals with at least 10 IIX fibers, and these subjects seemed to be less physically active compared with those individuals with <10 IIX fibers. Generalization of these results might only apply to moderately active males; however, also in subjects with <10 IIX fibers, the same trend in increased α-actinin-3 staining in IIX was observed. Furthermore, in the overall group (n = 90), h of physical activity per week did not differ between genotype groups, and including h of PA as a covariate did not fundamentally change the results of reported associations between absolute and relative dynamic torques.

Concerning the fiber type specific amount of α-actinin-2 protein, we found no differences in staining between the two genotype groups. Therefore, in XX-homozygotes no extra compensation seems to occur concerning the lack of α-actinin-3. Literature shows that α-actinin-2 and α-actinin-3 are structurally similar which makes it likely that α-actinin-2 is able to compensate for the lack of α-actinin-3 (13), as confirmed in the present study, however, with lower capacities of high velocity contractility.

To conclude, in the present study we expand the existing hypothesis that the R allele enhances high velocity muscle tasks to healthy young men (18–29 y). This study is the first to our knowledge to show an association between the ACTN3 (R577X) genotype and the fiber surface and number of fast glycolytic fibers (IIx), in terms of percentage in favor of the RR carriers. Fiber type specific α-actinin-3 content measures indicate a 17% higher staining intensity, indicative for a true difference in protein expression in type IIX fibers compared with type IIa fibers.

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REFERENCES


