

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

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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/jappphysiol.91435.2008.— $\alpha$ -Actinins are structural proteins of the Z-line. Human skeletal muscle expresses two  $\alpha$ -actinin isoforms,  $\alpha$ -actinin-2 and  $\alpha$ -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  $\alpha$ -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 modulate responsiveness to training. Our data further suggest that  $\alpha$ -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  $\alpha$ -actinin-3, which implies that  $\alpha$ -actinin-2 may compensate for the lack of  $\alpha$ -actinin-3 and hence counteract the phenotypic consequences of the deficiency.

*ACTN2*; exercise; physical performance; Wingate test

SKELETAL MUSCLE  $\alpha$ -actinins are actin-binding proteins that constitute the major structural component of the Z-line (3, 5). The two isoforms,  $\alpha$ -actinin-2 and  $\alpha$ -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,  $\alpha$ -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-bisphosphatase (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  $\alpha$ -actinin-2, can compensate for the lack of  $\alpha$ -actinin-3 protein. Interestingly, the frequency of the mutated X allele, and especially of the fully  $\alpha$ -actinin-3-deficient genotype (XX), is markedly lower in sprint and power athletes (26, 29, 30, 34). This may imply that the  $\alpha$ -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  $\alpha$ -actinin-3 aids in athletic events requiring superior aerobic capacity (26, 29, 34). Similarly, loss of  $\alpha$ -actinin-3 expression prompted a shift favoring aerobic metabolism in the knockout mouse model (6, 21, 22).

It has also been suggested that  $\alpha$ -actinins play a role in muscle fiber-type differentiation through interactions with the  $\text{Ca}^{2+}$ - 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  $\alpha$ -actinin isoforms may play similar roles, there are specific functions possessed by the  $\alpha$ -actinin-3 that are not compensated for by the  $\alpha$ -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  $> 30 \text{ kg/m}^2$ , training  $< 2 \text{ h/wk}$ , 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 in addition to the Wingate test performed a dynamic fatigue test (31) and maximal concentric knee extensions at different angular velocities. Muscle biopsies were obtained from vastus lateralis muscle at rest to assess fiber-type composition and to determine *ACTN2* and *ACTN3* mRNA levels. Subjects gave their informed consent to participate in the study, which was approved by the Human Ethics Committee of the Karolinska Institutet. The study was carried out in accordance with the Declaration of Helsinki of the World Medical Association.

### Subjects

Subjects were men ( $n = 61$ ) and women ( $n = 59$ ) with RR, RX, or XX genotype who 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 ( $> 3$ – $4 \text{ yr}$  of training;  $\sim 2$ – $10 \text{ h/wk}$ ), 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 ACGATCAGTTCAAGGCAACACT (forward) and ACCCTGGATGCCCATGATG (reverse), and the TaqMan probe sequences were TCGCTCTCGGTCAGC and CGCTCTCAGTCAGC (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

Sex:	ACTN3 Genotype						Statistics, <i>P</i> Value
	577 RR		577 RX		577 XX		
	Men ( <i>n</i> = 21)	Women ( <i>n</i> = 20)	Men ( <i>n</i> = 23)	Women ( <i>n</i> = 26)	Men ( <i>n</i> = 17)	Women ( <i>n</i> = 13)	All ( <i>n</i> = 120)
Age, yr	24±2	23±3	27±5	24±5	25±5	25±4	0.10
Weight, kg	79±11	66±9	81±10	64±8	78±7	61±8	0.30
BMI, kg/m <sup>2</sup>	24±3	23±3	24±2	23±2	23±2	22±2	0.15
BF, % ( <i>n</i> = 98)	16±6	28±5	18±5	28±7	14±4	31±6	0.47
FFW, kg ( <i>n</i> = 98)	66±7	48±6	67±10	46±5	67±6	46±11	0.96
Training, h/wk	5.2±3	5.6±2	5.6±3	5.0±2	4.5±3	5.0±2	0.56
PP, W/kg body wt	11.3±1	9.5±0.7	11.3±1	9.6±1	11.5±0.4	9.6±0.5	0.81
MP, W/kg body wt	10.1±1	8.6±0.7	10.3±0.6	8.7±0.9	10.2±0.4	8.6±0.4	0.78
FI-15, %	23±6	23±7	21±7	23±4	23±4	24±6	0.55
FI-30, %	46±6	42±5	45±9	43±6	46±4	43±5	0.91

Values are means  $\pm$  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

	<i>ACTN3</i> Genotype		Statistics, <i>P</i> Value
	577 RR ( <i>n</i> = 12)	577 XX ( <i>n</i> = 9)	
Age, yr	25 ± 2	26 ± 5	0.52
Weight, kg	85 ± 12	74 ± 7	0.03*
BMI, kg/m <sup>2</sup>	25 ± 3	23 ± 2	0.08
BF, %	16 ± 7	13 ± 4	0.34
FFW, kg	71 ± 6	65 ± 5	0.02*
Training, h/wk	4.3 ± 3	3.1 ± 2	0.36
MVC, N·m	329 ± 79	302 ± 41	0.36
Relative MVC, N·m/kg body wt	3.86 ± 0.7	4.08 ± 0.6	0.46
PT decrease 1–15, %	19 ± 7	19 ± 6	0.97
PT decrease 1–30, %	62 ± 7	63 ± 8	0.78

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.

power output during the 30-s duration of the sprint, were calculated and related to body mass. 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 a difference between PP and power at 30 s as a percentage of PP (FI-30).

**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 (Muscle-lab, 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.

#### Muscle Biopsies

Needle biopsy technique (4) was used to obtain samples from the quadriceps femoris vastus lateralis muscle. Muscle specimens for determination of fiber-type composition were frozen in isopentane precooled with liquid nitrogen and stored at –70°C until analysis. Frozen muscle samples were mounted in embedding medium, and 10-μm transverse sections were cut and prepared for histochemical staining. Muscle samples for extraction of total RNA were frozen in liquid nitrogen immediately after extrication.

#### 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 IIc 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 Hs01552477\_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  $2^{-(Ct, \text{housekeeping gene} - Ct, \text{target gene})}$ . 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-Winberg 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-Winberg 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



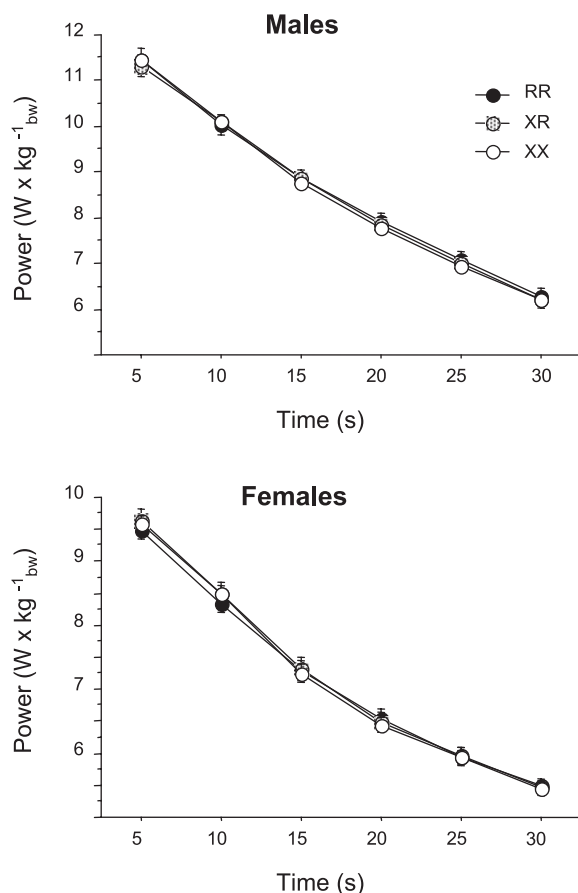


Fig. 1. Power output profiles normalized for body weight (BW) during 30-s Wingate cycling in men and women with different *ACTN3* R577X genotypes. Values shown are means and SEs.

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  $\times$  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  $\pm$  SD)  $55 \pm 12$ ,  $57 \pm 12$ , and  $54 \pm 17$ ; of type IIa was  $32 \pm 12$ ,  $34 \pm 10$  and

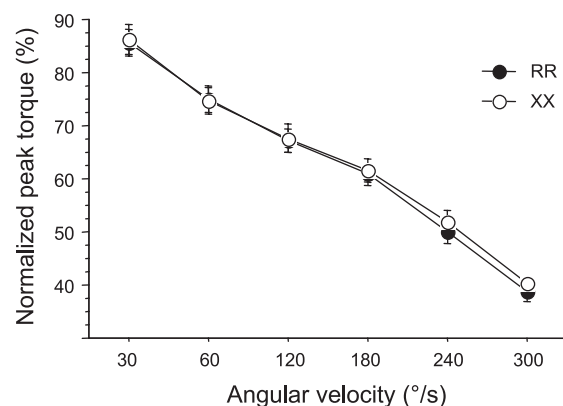


Fig. 2. Knee extensor force-velocity relationship during isokinetic exercise in men with different *ACTN3* R577X genotypes. Values shown are means and SEs.

$35 \pm 14$ ; and of type IIb was  $12 \pm 10$ ,  $8 \pm 5$ , and  $11 \pm 12$  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  $\beta$ -actin (Fig. 4). The *ACTN3* mRNA level was (mean  $\pm$  SD)  $0.40 \pm 0.4$  AU in XX and more than 10-fold higher in RR ( $6.98 \pm 5.5$  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

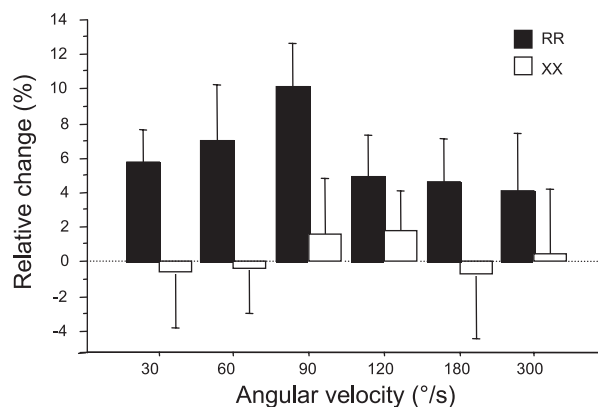


Fig. 3. Change in relative peak torques, measured on 2 occasions 1 wk apart, in men with different *ACTN3* R577X genotypes. Values shown are means and SEs. Repeated-measures ANOVA:  $P = 0.055$ .

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

Sex:	ACTN3 Genotype						Statistics, <i>P</i> Value
	577 RR		577 RX		577 XX		
	Men ( <i>n</i> = 16)	Women ( <i>n</i> = 7)	Men ( <i>n</i> = 9)	Women ( <i>n</i> = 8)	Men ( <i>n</i> = 12)	Women ( <i>n</i> = 11)	
Type I, %	49±17	66±7	54±10	62±13	52±14	59±8	0.73
Type IIa, %	39±14	25±8	36±6	31±13	31±15	34±10	0.79
Type IIb, %	12±13	8±5	9±5	7±6	16±10	7±8	0.49

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

( $r = 0.507$ ;  $P = 0.112$ ). The *ACTN2* mRNA level was markedly higher than that of *ACTN3*, and there was no difference in *ACTN2* expression between XX ( $296 \pm 182$ ) and RR ( $246 \pm 136$ ). 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 nonexistent 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 ( $\sim 10\%$ ) 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 concurs with a recent study by McCauley and co-workers (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

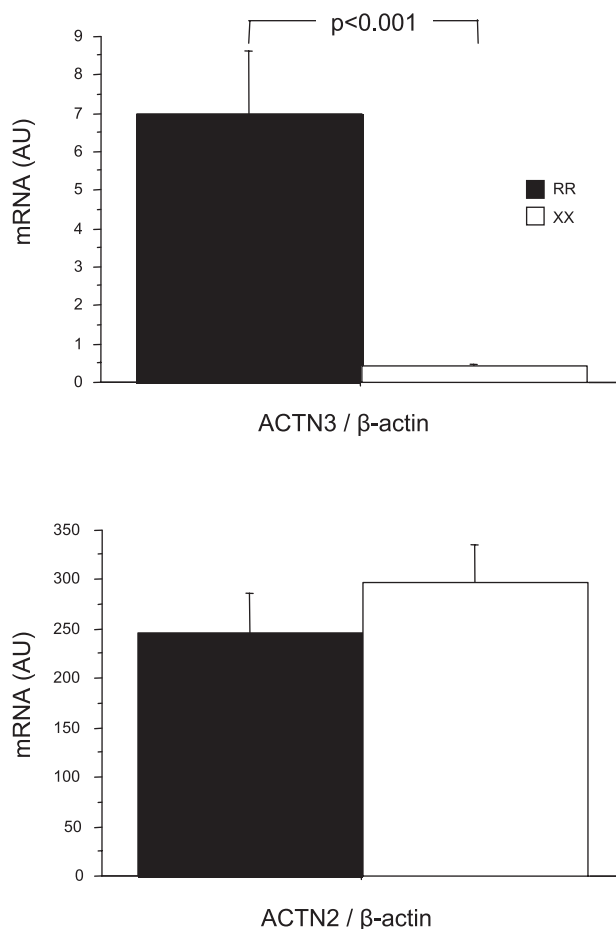


Fig. 4. *ACTN3* and *ACTN2* mRNA levels in men and women with different *ACTN3* R577X genotypes. Values are expressed in arbitrary units (AU) corrected to  $\beta$ -actin and shown as means and SEs. The *ACTN3* mRNA level was significantly higher in RR than in XX:  $P < 0.001$ .

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  $\alpha$ -actinin-3 influences fiber-type differentiation by promoting glycolytic type II fibers, while lack of  $\alpha$ -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  $\alpha$ -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  $\alpha$ -actinin-3 in the XX genotype seems not to compromise muscle function, suggesting that the other isoform, the  $\alpha$ -actinin-2, may compensate for the lack of  $\alpha$ -actinin-3 protein. The present study aimed at exploring the interrelation 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  $\alpha$ -actinin-2 (24, 33). In addition, our results demonstrate that the expression of *ACTN2* is affected by the content of  $\alpha$ -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  $\alpha$ -actinin-2 compensates for the lack of  $\alpha$ -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  $\alpha$ -actinin-3 protein. The  $\alpha$ -actinins are composed of two antiparallel subunits assembled into homodimers (5). The two  $\alpha$ -actinin isoforms are coexpressed in type II fibers and may be assembled into  $\alpha$ -actinin-3/2 heterodimers (7). Downregulation of the *ACTN3* mRNA expression protects skeletal muscle from the potentially harmful effects of the defective  $\alpha$ -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  $\alpha$ -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  $\alpha$ -actinin-2 expression is more substantial than the expression of  $\alpha$ -actinin-3. Hence, even small increases in the  $\alpha$ -actinin-2 content may be sufficient to compensate for deficiency of  $\alpha$ -actinin-3. Such small differences in the content of  $\alpha$ -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  $\alpha$ -actinins 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  $\alpha$ -actinin-3, which implies that  $\alpha$ -actinin-2 may compensate for the lack of  $\alpha$ -actinin-3 and hence counteract the phenotypic consequences of the deficiency.

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