ACTN3 R577X polymorphism and neuromuscular response to resistance training

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Abstract
The R577X polymorphism at the ACTN3 gene has been associated with muscle strength, hypertrophy and athletic status. The X allele, which is associated with the absence of ACTN3 protein is supposed to impair performance of high force/velocity muscle contractions. The purpose of the present study was to investigate the association of the R577X polymorphism with the muscle response to resistance training in young men. One hundred forty one men performed two resistance training sessions per week for 11 weeks. Participants were tested for 1RM bench press, knee extensors peak torque, and knee extensors muscle thickness at baseline and after the training period. Genotyping was conducted using de DdeI restriction enzyme. Genotype distribution was 34.4% for RR, 47% for RX and 18.6% for the XX genotype. According to the results, the R577X polymorphism at the ACTN3 gene is not associated with baseline muscle strength or with the muscle strength response to resistance training. However, only carriers of the R allele showed increases in muscle thickness in response to training.

Key words: Muscle strength, muscle hypertrophy, peak torque, genotype, alpha-actinin 3, knee extensor.

Introduction
Muscle strength has an important influence on functional abilities and has been positively associated with sports performance (ACSM, 2009; Jung, 2003), longevity, and quality of life (Newman et al., 2006; Visser et al., 2005). Although environmental stimuli are known to be important determinants of muscle strength and size, inheritance has an important influence on these phenotypes (Bray et al., 2009; Stewart and Rittweger, 2006; Thomis et al., 1997). It is estimated that genetic factors may correspond to 44 to 58% of the inter individual variations in muscle strength (Beunen and Thomis, 2004; Tiainen et al., 2009) and lean mass (Arden and Spector, 1997) and this influence is more evident in young than older people (Prior et al., 2007; Stewart and Rittweger, 2006). In this regard, one of the genes that may influence these phenotypes is the alpha-actinin 3 (ACTN3) gene (Clarkson et al., 2005; Niemi and Majamaa, 2005; Yang et al., 2003).

The ACTN3 is a protein specific to the type 2 fibers (Beggs et al., 1992; North et al., 1999) located at the Z disks and has important functions in muscle metabolism and structure (Beggs et al., 1992; MacArthur and North, 2004, 2007). The ACTN3 gene is located in the 11q13-q14 chromosomal band and was first cloned by Beggs et al. (1992). Later, a functional polymorphism at the ACTN3 gene was identified in humans by North et al. (1999). This mutation, known as R577X (rs1815739), is a transition from Cytosine (R allele) to Thymine (X allele) at position 1747 in the exon 16, resulting in a premature stop codon, which is associated with the absence of ACTN3 expression in carriers of the XX genotype (North et al., 1999).

The XX genotype does not cause any apparent phenotype or histological changes, suggesting that the presence of the protein is not critical (North and Beggs, 1996) and the variations in muscle function occurs within the normal range (MacArthur et al., 2008). Analyses in rats revealed that the lack of ACTN3 lead to loss of muscle mass, associated with reductions in the cross-sectional area of muscles composed primarily by type 2 fibers (Chan et al., 2008; MacArthur et al., 2008). Additionally, fibers of animals with the XX genotype have lower work capacity and longer twitch half relaxation time (Chan et al., 2008; MacArthur et al., 2008).

Previous studies have shown that the XX genotype is underrepresented in strength and power athletes relative to controls and endurance athletes (Druzhkevskaia et al., 2008; Eynon et al., 2009; Niemi and Majamaa, 2005; Papadimiroiu et al., 2008; Roth et al., 2008; Yang et al., 2003; Yang et al., 2007). On the basis of the physiological function of ACTN3 and the relationship between polymorphic forms of ACTN3 gene and exercise performance, it is expected that the R577X polymorphism is associated with baseline muscle size, strength and power, however, the results of cross-sectional studies are controversial (Clarkson et al., 2005; Delmonico et al., 2007; Delmonico et al., 2008; McCauley et al., 2009; Moran et al., 2007; Norman et al., 2009; San Juan et al., 2006; Walsh et al., 2008). Studies in older (Delmonico et al., 2008; San Juan et al., 2006; Walsh et al., 2008) and younger (McCauley et al., 2009; Moran et al., 2007; Norman et al., 2009) people revealed no differences in muscle strength and power among different R577X genotypes. However, some separated analyses in women revealed that carriers of the XX genotype have lower muscle strength and power (Clarkson et al., 2005; Delmonico et al., 2008; Walsh et al., 2008) while others reported that the XX genotype group have greater muscle power than carriers of the R allele (Delmonico et al., 2007).

Although it is widely known that genetic factors may influence muscle strength response to resistance training, the determination of the specific genes associated with this response is still in its infancy. With regard to ACTN3, Delmonico et al. (2007) reported that older women, but not men, with the RR genotype had greater increases in muscle power after 10 weeks of unilateral knee extensor resistance training. No difference in 1RM gains among genotypes were seen in either gender. The
only known study with young subjects was published by Clarkson et al. (2005). The authors studied associations between ACTN3 genotype and elbow flexor strength in young men and women enrolled in 12 weeks of resistance training. It was reported that women carrying the X allele had greater 1RM gains compared to the RR homozygotes, but the results in men were not significant (Clarkson et al., 2005). Therefore, it is not known if the R allele would favor muscle adaptations due to its physiological function or if the carriers of the X allele would be favored by the “principle of the initial value”, which determines that subjects with lower baseline values show an increased response to training, as suggested by Clarkson et al. (2005). Due to the controversy in the literature as well as the scarcity of experimental studies investigating the effects of R577X in muscle strength and hypertrophy in response to training, the purpose of the present study was to investigate the influence of the R577X polymorphism at the ACTN3 gene on the muscle response to resistance training in young men.

Methods

Study overview
The study investigated the association of the ACTN3 R577X genotype with neuromuscular response to resistance training in 141 young men. Before, and 5-7 days after 11 weeks of training, 1RM bench press and knee extensors concentric peak torque were obtained from all volunteers. Resistance training sessions comprised two sets of 8-12 repetitions of five exercises and were designed to exercise the major muscle groups. All testing and training sessions were conducted at the same time of the day. For genotype analysis, 4 ml of blood was collected from the antecubital vein after the final 1RM bench press test. Genotyping was done using a procedure that involved the DdeI restriction enzyme.

Participants
Two hundred and ten college aged men volunteered to participate in the study. Volunteers were selected at random from respondents to fliers distributed over the university campus, and by word-of-mouth. The criteria for entering the study included being at least 18 years of age, no previous resistance training experience and being free of clinical problems that could be aggravated by the study procedures. To be included in the study, subjects had to attend at least 90% of the training sessions. The volunteers were oriented to not change their nutritional habits during the study period, and if any relevant change was detected (i.e. becoming a vegetarian, restricting calories, taking nutritional supplements or ergogenic aids, etc.) participants’ data were excluded from the analysis. At the end of the study, 141 subjects met the criteria for entering the study (22.0 ± 2.7 years; 1.75 ± 0.07 m; 72.0 ± 13.9 kg). Most of the exclusions were due to performance of strength training other than the study protocol, changes in nutritional habits and low training attendance. The excluded subjects were similar to the others with regard to genotype distribution and physical characteristics. The study has been performed in accordance with the ethical standards of the International Journal of Sports Medicine (Harris and Atkinson, 2009). All participants were notified of the research procedures, requirements, benefits and risks before providing written informed consent. The Institutional Research Ethics Committee granted approval for the study.

Procedures

One repetition maximum test
In the week before the experiment and 5-7 days after the last training session, the load for 1RM was determined for each subject in the bench press exercise. The test protocol recommended by Kraemer and Fry (1995) was adopted as follows: 1. A warm-up involved 5-10 repetitions at 40-60% of the estimated 1RM, 2. One minute to rest with light stretching followed with 3-5 repetitions at 60-80% of the estimated 1RM, 3. Three to five attempts to reach the 1-RM with 5-minute rest intervals between each new lift. The maximum weight that was successfully lifted was recorded. Verbal encouragement was given during the trials to obtain the best performance. The initial tests were repeated in all subjects, and the intraclass correlation coefficient (ICC) was 0.98.

Measurement of isokinetic concentric peak torque (PT)
Knee extensor isokinetic concentric PT was measured on a Biodex System 3 Isokinetic Dynamometer (Biodex Medical, Inc., Shirley, NY). Calibration of the dynamometer was performed according to the manufacturer’s specifications before every testing session. The subjects sat upright with the axis of rotation of the dynamometer arm oriented with the lateral femoral condyle of the right knee. Belts were used to secure the thigh, pelvis, and trunk to the dynamometer chair to prevent additional body movement. The chair and dynamometer settings were recorded to ensure the same positioning for all tests. Gravity correction was obtained by measuring the torque exerted on the dynamometer resistance adapter with the knee in a relaxed near full extension. The tests comprised two sets of five repetitions at 60º/s. Subjects were instructed to fully extend and flex the knee and to work maximally during each set. Verbal encouragement was given throughout the test session. After each set, subjects were required to take 60 s of rest before the onset of the next set. The knee strap was released during each rest period to ensure unrestricted blood flow to the lower limb. The procedures were administered to all subjects by the same investigator. Knee extensor PT baseline test and retest ICC and standard error of the mean (SEM) were 0.98 and 2.3 % respectively.

Muscle thickness (MT)
Participants were tested before and after the 11-week training period for MT of the knee extensors of the right limb. All tests were conducted at the same time of the day, and participants were instructed to hydrate normally 24 hours before the tests. Measures were taken 3-5 days after the last training session to prevent any swelling from contributing to the MT measurement (Chilibeck et al., 2004). During this time, participants were instructed to not participate in any other exercise sessions or intense activity. MT was measured using B-Mode ultrasound
Genotyping
DNA was extracted using a salting out protocol. Blood samples were obtained from the antecubital veins of all volunteers in tubes containing EDTA anti-coagulant. Genomic loci containing the polymorphic site was amplified using PCR. The primers for PCR (5’-CCCACAACTTTAGGCTCCTG-3’ – forward and 5’-ATGTAGGGATTGTTGAGCCA-3’ – reverse) were designed using the Primer3Plus software, available on the internet (http://www.bioinformatics.nl/cgi-bin/primer3plus/prime3plus.cgi). The 342pb amplified fragment subsequently underwent digestion by DdeI (Promega, Madison, WI, EUA). The digested products were then electrophoresed in a 2% agarose gel and then visualized in UV light. Visual analysis of the gel resulted in three possible results: RR (342pb), RX (342, 250, 92pb) or XX (250, 92pb). The accuracy of the genotyping assay was verified by using positive and negative control samples previously genotyped using the same method, following previous recommendations (Chanock et al., 2007)

Statistical analyses
Distribution of ACTN3 R577X genotypes was analyzed by Chi-square to verify agreement with Hardy-Weinberg equilibrium. To test for differences among genotypes in age, height, weight, initial bench press 1RM and knee extensor PT and MT, one way Analysis of Variance (ANOVA) was performed. To examine the association between the ACTN3 R577X genotypes and the RT-induced adaptations, a repeated measures ANOVA (genotype x time) was performed, in which the within-subjects factors were pre and post values of the phenotypes under study and the between subjects factors were the genotypes (RR, RX and XX). Whenever necessary, multiple comparisons with confidence interval adjustment by the Bonferroni procedure were used as post hoc. Relative percentage change was calculated for the knee extensor MT, age, height, weight, initial bench press 1RM and knee extensor PT and MT, one way Analysis of Variance (ANOVA) was performed. To examine the association

Results

Characteristics of the subjects, according to the ACTN3 R577X genotype are presented in Table 1. Genotype

Table 1. Subjects characteristics according to the ACTN3 R577X genotypes. Values are means (± standard deviation).

<table>
<thead>
<tr>
<th>Variables</th>
<th>RR (n = 50)</th>
<th>RX (n = 66)</th>
<th>XX (n = 25)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>21.9 (2.1)</td>
<td>21.8 (2.6)</td>
<td>22.6 (4.3)</td>
<td>.265</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.76 (.07)</td>
<td>1.75 (.07)</td>
<td>1.74 (.06)</td>
<td>.210</td>
</tr>
<tr>
<td>Body mass (kg)</td>
<td>73.1 (13.7)</td>
<td>71.5 (14.8)</td>
<td>70.4 (12.6)</td>
<td>.716</td>
</tr>
<tr>
<td>Body mass index (kg·cm⁻²)</td>
<td>23.5 (4.0)</td>
<td>23.3 (3.8)</td>
<td>23.3 (3.6)</td>
<td>.959</td>
</tr>
<tr>
<td>Knee extensors PT (Nm)</td>
<td>229 (43)</td>
<td>223 (35)</td>
<td>224 (27)</td>
<td>.608</td>
</tr>
<tr>
<td>Delta variation (%)</td>
<td>6.1</td>
<td>6.9</td>
<td>7.7</td>
<td>.926 **</td>
</tr>
<tr>
<td>Bench press 1RM (kg)</td>
<td>60.2 (14.6)</td>
<td>59.1 (12.7)</td>
<td>59.5 (17.5)</td>
<td>.909</td>
</tr>
<tr>
<td>Delta variation (%)</td>
<td>11.1</td>
<td>12.3</td>
<td>9.6</td>
<td>.282 **</td>
</tr>
</tbody>
</table>

PT= peak torque; 1RM= one repetition maximum; *p < 0.05 between pre and post-training; **time by genotype interaction
Table 2. Subjects characteristics according to the presence of the R allele. Values are means (± standard deviation).

<table>
<thead>
<tr>
<th>Variables</th>
<th>R (n= 116)</th>
<th>XX (n = 25)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>21.8 (2.4)</td>
<td>22.6 (4.3)</td>
<td>.234</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.76 (.07)</td>
<td>1.74 (5.6)</td>
<td>.196</td>
</tr>
<tr>
<td>Body mass Pre (kg)</td>
<td>72.2 (14.3)</td>
<td>70.4 (12.6)</td>
<td>.579</td>
</tr>
<tr>
<td>Body mass Post (kg)</td>
<td>73.3 (15.6)</td>
<td>71.0 (10.6)</td>
<td>.587</td>
</tr>
<tr>
<td>Body mass index Pre (kg·cm⁻²)</td>
<td>23.4 (3.9)</td>
<td>23.3 (3.6)</td>
<td>.915</td>
</tr>
<tr>
<td>Body mass index Post (kg·cm⁻²)</td>
<td>23.7 (4.1)</td>
<td>23.2 (2.6)</td>
<td>.659</td>
</tr>
<tr>
<td>Knee extensors PT Pre (Nm)</td>
<td>225 (39)</td>
<td>224 (27)</td>
<td>.868</td>
</tr>
<tr>
<td>Knee extensors PT Post (Nm)</td>
<td>240 (39) *</td>
<td>241 (33) *</td>
<td>.917</td>
</tr>
<tr>
<td>Delta variation (%)</td>
<td>6.6</td>
<td>7.7</td>
<td>.996 **</td>
</tr>
<tr>
<td>Bench press 1RM Pre (kg)</td>
<td>59.6 (13.5)</td>
<td>59.5 (17.5)</td>
<td>.983</td>
</tr>
<tr>
<td>Bench press 1RM Post (kg)</td>
<td>66.6 (12.2) *</td>
<td>65.2 (13.1) *</td>
<td>.611</td>
</tr>
<tr>
<td>Delta variation (%)</td>
<td>11.8</td>
<td>9.6</td>
<td>.121 **</td>
</tr>
</tbody>
</table>

PT= peak torque; 1RM= one repetition maximum; *p < 0.05 between pre and post-training; **time by genotype interaction
distribution was in Hardy-Weinberg equilibrium ($X^2 = 0.726$ p > 0.05). According to the results, 35.5% of the volunteers were classified as RR, 46.8% as RX and 17.7% as XX. Overall compliance to the training program was 93% and it did not differ among genotypes.

There were no differences among genotypes for baseline values of knee extensors PT and bench press 1RM (p > 0.05). The ANOVA for PT and 1RM revealed no significant interaction of group by time (p > 0.05). However, a significant main effect for time was found for PT and 1RM. When main effect of time was analyzed, all genotypes significantly increased knee extensors PT and bench press 1RM (p < 0.05). Additional comparisons between carriers of the R allele (RR + RX) and the XX genotype (Table 2) revealed no difference between groups for baseline values and changes in knee extensors PT and bench press 1RM (p > 0.05).

Discussion

Genotype distribution in the present study was similar to previously reported in Caucasians (Clarkson et al., 2005; Delmonico et al., 2007; 2008; Druzhevskaya et al., 2008; Eynon et al., 2009; Moran et al., 2007; Niemi and Majamaa, 2005; Paparini et al., ; Walsh et al., 2008) and Hispanics (Clarkson et al., 2005), but different from the distribution reported in Asians and African-Americans, who present a increased and decreased frequency of the X allele, respectively (Clarkson et al., 2005; North and Beggs, 1996). In the present study, we tested whether the R577X polymorphism was associated with baseline muscle strength and MT and their response to resistance training in a group of untrained young men. Bench press 1RM, knee extensors PT and knee extensors MT were measured before and 11 weeks after a resistance training protocol.
We found that baseline values of muscle strength and MT were not different among R577X genotypes, which is in agreement with analyses conducted in older and young men (Delmonico et al., 2007; 2008; McCauley et al., 2009; Norman et al., 2009; San Juan et al., 2006).

The known analysis that reported differences in muscle strength and power among ACTN3 genotypes were done in women (Clarkson et al., 2005; Delmonico et al., 2007; Walsh et al., 2008), suggesting that the effects of the R577X polymorphism may be gender dependent. One possible explanation for this gender dependent effect is that the anabolic effect of the male steroid hormones could overlap the effects of muscle strength and power gains as a result of the ACTN3 protein in muscle structure (MacArthur and North, 2004).

With regard to muscle strength and power response to resistance training, Clarkson et al. (2005) studied the association of R577X genotypes with muscle phenotypes in young men and women before and after 12 weeks of resistance training. Training was conducted two days per week and directed to the elbow flexors and extensors of the non-dominant side. According to the results, women bearing the X allele showed greater 1RM increases than carriers of the RR genotype. However, the results in men did not achieve significance. Later, Delmonico et al. (2007) examined the effects of the polymorphism R577X in muscle power changes after 10 weeks of resistance training in older women and men. Training was performed three times per week and was composed of exercises for the knee extensors and flexors. Contrary to the results reported by Clarkson et al. (2005), women with the RR genotype had greater increases in muscle power, but the results in men did not achieve significance. There were no differences in IRM increase between genotypes for men or women. The reason for the differences between the results of Delmonico et al. (2007) and Clarkson et al. (Clarkson et al., 2005) may be related to the characteristics of the groups, since in both studies, the group with lower initial values showed the greater increases, independently of the genotypes. Therefore, the results may be related to an increased possibility of improvement in persons with lower initial values and not to variations in the ACTN3 genotype.

It is important to note that in the studies of Clarkson et al. (Clarkson et al., 2005) and Delmonico et al. (2007) there were no difference for the changes in performance among the genotypes in men. Therefore, our results confirm previous studies in men (Clarkson et al., 2005; Delmonico et al., 2007). Although the present and other experimental and cross-sectional studies suggest that the R577X polymorphism does not influence muscle phenotypes in men, the fact that XX genotype is under-represented in strength and power athletes (Drushedevskaya et al., 2008; Eynon et al., 2009; Niemi and Majamaa, 2005; Papadimitriou et al., 2008; Roth et al., 2008; Yang et al., 2003; 2007) is intriguing. If the success of the athletes involved in sports that require high force, high intensity muscle contractions seems to be related to the presence of the ACTN3 protein, it is possible to suggest that the R allele is advantageous for generating force and velocity, and that this allele may be associated with a better response to training. However, this was not seen in the present study.

One possible explanation is that, in non-athletes, the difference derived from the R577X polymorphism, when analyzed alone, is too low to be relevant, especially in young people, as suggested by McCauley et al. (2009). Previous analyzes have suggest that the R577X genotype are responsible for ~1-2% of the variations in muscle strength (Clarkson et al., 2005; Walsh et al., 2008), although this difference seems relative small in daily live activities, it may be the difference between winning or losing a competition in a high competitive context. Moreover, to a high level athlete, it is necessary to have a combination of favorable genes in order to be successful, and the ACTN3 may be one of these many genes. Therefore, when we analyze athletes, we are probably not just analyzing carriers of the R allele, instead, we are studying carriers of a favorable genetic pool and, among them, is the R577X genotype. On the other hand, this may not be the case when we study a sample of non-athletes, which may have heterogeneous genetic characteristics, such that the R allele is not accompanied by other favorable genetic variants.

Additionally, the intensity and volume of the training sessions performed in this and other studies (Clarkson et al., 2005; Delmonico et al., 2007) may have been too low in comparison to the training performed by athletes. Athletes usually train many hours a day for many years, while the available studies are no longer than 12 weeks and did not involve more than 3 hours of training per week. This low volume and short duration training could not have been enough to induce the manifestation of the functional differences among the R577X genotypes.

With regard to the effects of the ACTN3 gene variations in muscle hypertrophy in response to training, Clarkson et al. (Clarkson and Newham, 1995) did not find differences among the R577X genotypes in muscle mass response to 12 weeks of resistance training in young men and women. In agreement to Clarkson and Newham (1995) results, we also did not find differences among genotypes in the present analyses. However, it is important to mention that the carriers of the R allele showed significant increases in MT in response to training.

The fact that the absence of the R allele impairs muscle hypertrophy in response to training could be due to the functional effects of ACTN3. Yang et al. (2003) initially suggested that the ACTN3 protein promotes formation of type II fibers. Later, Vincent et al (Vincent et al., 2007) demonstrated that carriers of the XX genotype had a decreased percentage of type IIx fibers in comparison with carriers of the R allele, although this is not supported by the study Norman et al. (Norman et al., 2009). If the XX genotype is, in fact, associated with a lower proportion of fast fiber type, it is comprehensible that this group show an impairment in hypertrophy, because previous studies showed that type I fibers hypertrophy less than type II fibers in response to training (Fry, 2004; Hather et al., 1991; Tesch and Karlsson, 1985).

**Conclusion**

The present study found that, in young men, the R577X polymorphism at the ACTN3 gene does not influence
baseline muscle strength, MT or the short-term muscle strength response to 11 weeks of low volume resistance training. On the other hand, besides there was no interaction among genotypes, the results suggested that the presence of the R allele may have some influence in muscle hypertrophy response to resistance training. These results however should be confirmed in other contexts, such as in a sample of women, higher volume and/or higher intensity training protocols and longer training periods.

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References


Key points

• ACTN3 Genotype distribution in the present study was similar to others populations (34.4% for RR, 47% for RX, and 18.6% for the XX).

• The R577X polymorphism at the ACTN3 gene is not associated with baseline muscle strength or with the muscle strength response to resistance training.

• It appears that the R allele carriers respond better to muscle thickness gains in response to training.

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