Genetic and Environmental Determinants of Healthy Aging

Association of the ACTN3 Genotype and Physical Functioning With Age in Older Adults

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Objective. The purpose of this study was to examine the association of the alpha-actinin-3 (ACTN3) R577X polymorphism on muscle function and physical performance in older adults.

Methods. We measured knee extensor torque, midthigh muscle cross-sectional area, muscle quality, short physical performance battery score, and 400-meter walk time at baseline and after 5 years in white older adults aged 70–79 years in the Health, Aging and Body Composition Study cohort (n = 1367). Incident persistent lower extremity limitation (PLL) over 5 years was additionally assessed. We also examined white men in the Osteoporotic Fractures in Men Study, a longitudinal, observational cohort (n = 1152) of men 65 years old or older as a validation cohort for certain phenotypes.

Results. There were no significant differences between genotype groups in men or women for adjusted baseline phenotypes. Male X-homozygotes had a significantly greater adjusted 5-year increase in their 400-meter walk time compared to R-homozygotes and heterozygotes (p = .03). In women, X-homozygotes had a ~35% greater risk of incident PLL compared to R-homozygotes (hazard ratio = 0.65, 95% confidence interval = 0.44–0.94). There were no other significant associations between any of the phenotypes and ACTN3 genotype with aging in either cohort.

Conclusions. The ACTN3 polymorphism may influence declines in certain measures of physical performance with aging in older white adults, based on longitudinal assessments. However, the influence of the ACTN3 R577X polymorphism does not appear to have a strong effect on skeletal muscle–related phenotypes based on the strength and consistency of the associations and lack of replication with regard to specific phenotypes.

Key Words: Genetics—Elderly—Sarcopenia—Skeletal muscle.

TWIN studies indicate that muscle function phenotypes are highly heritable (1,2), and various candidate genes have been studied to explain some of the high inter-individual variability observed in these phenotypes (3). However, only a small number of genes containing single nucleotide polymorphisms (SNPs) have been identified as candidate genes for explaining this variability.

Alpha-actinin-3 (ACTN-3), a structural protein in type II skeletal muscle fibers, is associated with physical performance (4–8). Recent data suggest that the R577X SNP in the ACTN-3 gene (ACTN3) explains some of the inter-individual variability in muscle functioning in older adults (9), although not all studies agree (10). The R577X SNP results from a C-to-T transition at position 1747 in exon 16 that substitutes an arginine residue at codon 577 for a premature stop codon (11). X-homozygotes fail to express ACTN-3, but this is not known to be associated with any disease phenotype (12). Nevertheless, the lack of ACTN-3 expression has been associated with athletic performance (5,13).

Although few studies have been reported that have examined the association of the ACTN3 R577X polymorphism in older adults, studies in younger athletes show that ACTN-3 is associated with better performance in athletes who are engaged in muscle power–dependent sports (5,7,8,13). In addition, data from cross-sectional studies show that young adult X-homozygotes have lower strength...
than heterozygotes (4) and R-homozygotes (14). Moreover, we recently reported that the R577X polymorphism is associated with muscle power response to strength training in older adults (9). However, there were no differences in muscle size, strength, or muscle power at baseline; these findings agreed with those of a previous report (10). Nevertheless, that study was limited by a relatively small sample size.

The influence of the ACTN3 R577X polymorphism on skeletal muscle and physical functioning in older adults has not been thoroughly examined in a large epidemiologic cohort. Additionally, no reports have addressed the potential impact of this polymorphism on age-related changes in these phenotypes. Thus, the purpose of this study was to determine the association of the ACTN3 R577X polymorphism with muscle traits and physical functioning in a large cohort of older adults and to determine the association of this polymorphism with age-related declines in these phenotypes.

**METHODS**

**Participants**

The Health, Aging and Body Composition (Health ABC) Study is a longitudinal, observational study of 3075 well-functioning men and women between the ages of 70 and 79 years. White participants were recruited in 1997–1998 from a random sample of Medicare enrollees in Memphis, Tennessee, and Pittsburgh, Pennsylvania. Fifty-two percent of the participants were women, and 41% were black. Eligible participants had no self-reported difficulty walking one-quarter mile, climbing 10 steps, and performing activities of daily living; did not report using a walking aid; and were free of cancer under active treatment. Each institutional review board (IRB) approved the protocol, and all participants gave written informed consent for study participation.

Although we performed preliminary analyses on all participants, there was a very strong racial influence on many of the phenotypes of interest in both men and women. Combining black and white participants appeared to result in spurious associations between the association of the ACTN3 R577X polymorphism and some skeletal muscle phenotypes. This racial difference in skeletal muscle phenotypes has previously been observed in the Health ABC cohort (15). Moreover, of the 868 black participants, there were only 38 X-homozygotes for baseline analyses and 19 X-homozygotes for 5-year changes. The exclusion of blacks from the present analysis resulted in a sample size of 1794 white participants.

Of the 1794 remaining participants, some were excluded from the present analysis because of missing genotype (n = 234) or phenotype (n = 193) data. Thus, baseline analysis was conducted on the remaining 1367 participants (726 men and 641 women) who had complete data. Because these measures required a 5-year follow-up visit, there was some loss to follow-up (n = 372).

A second cohort of men from the Osteoporotic Fractures in Men (MrOS) Study was also studied. The MrOS Study is a multicenter, observational, longitudinal study of 5995 community dwelling, ambulatory men 65 years old or older (16,17). To be eligible, men had to be able to give informed consent, walk without assistance, not have a bilateral hip replacement, be able to give self-reported data, and not have a medical condition that would have resulted in imminent death (16,17). A subset of men (n = 1201) from the Pittsburgh and Minneapolis centers form the basis of the current analysis. Although there were some nonwhite participants in the current MrOS sample (n = 49), including only one X-homozygote, we decided to exclude these participants because of their small number. The MrOS Study was approved by the University of Pittsburgh and the University of Minnesota IRBs, and written informed consent was obtained from all participants. The MrOS Study included a 4-year follow-up visit, so there was some loss to follow-up (n = 124).

**Outcomes—Health ABC Study**

**Thigh cross-sectional area.**—In the Health ABC Study cohort, midhigh cross-sectional area (CSA) of the right leg was measured at baseline and after 5 years at the level of the midfemur using computed tomography (CT) as previously described (18). Briefly, a single 10 mm-thick axial image (120 kVp, 200–250 mAs) of the right thigh was acquired at the midpoint of the distance between the medial edge of the greater trochanter and the intercondylar fossa. The total area of nonadipose and nonbone tissues within fascial border was used as a quantification of muscle area (cm²).

**Knee extensor torque.**—Average isokinetic knee extensor muscle torque (MT, N-m), was measured at baseline and after 5 years using a Kin-Com 125 AP Dynamometer (Chattanooga, TN) at 60 degrees per second on the right leg, and the mean of the three most replicable and satisfactory trials of a limit of six attempts was used. When MT of the right leg was not available or valid because of knee replacement or pain, MT of the left leg was used in all analyses and was matched with the left leg thigh CSA for calculation of muscle area and muscle quality. Muscle quality was defined as MT/midhigh muscle CSA and was calculated at baseline and after 5 years.

**Self-reported functional limitation.**—Incident persistent lower extremity limitation (PLL) was assessed and defined by self-reported difficulty walking one-quarter mile or climbing 10 steps without resting at two consecutive 6-month intervals. Incident PLL was assessed over a 5-year follow-up period by telephone interviews and annual clinic visits. Because of the study exclusion criteria, no participants had baseline lower extremity limitation.

**Physical performance.**—Physical performance was evaluated at baseline and after 5 years using the Established Populations for Epidemiologic Studies of the Elderly (EPESE) short physical performance battery (SPPB) using previously described methods (19,20). The SPPB included five chair stands and tests of gait speed and standing balance.
In addition, participants were also asked to walk 400 meters after a 2-minute warm-up using methods described elsewhere (21). Standard vocal support was given during the test, and time to complete the test(s) was recorded.

Outcomes—MrOS

In the MrOS cohort, thigh fat-free mass (FFM, kg) was measured using dual-energy x-ray absorptiometry (QDR 4500; Hologic, Waltham, MA). Physical performance was assessed during the baseline examination, during a single baseline visit. Rigorous centralized training, examiner certification in protocol administration, and periodic protocol review during the course of the study were used to ensure consistency in the measures of physical performance.

Grip strength (kg) was measured in both hands using a Jamar handheld dynamometer (Sammons Preston Rolyan, Bolingbrook, IL) (22). The maximum effort from two trials of both hands was analyzed. Participants with a recent injury or new weakness in the hands or wrists could elect to skip this assessment, in which case they were considered unable to complete the grip strength assessment.

Leg power (W) was measured using the Nottingham Power Rig (23,24). Participants completed up to nine measurements on each leg separately; the overall maximal leg power from both legs was analyzed. In an analysis of a small subsample of the MrOS participants (n = 55), test–retest reliability was estimated. Coefficients of variation (CVs) for between-examiner consistency ranged from 2.6% to 3.5%, and the CVs representing the combination of within-examiner variance, within-participant variance, and machine variance were <11%. Leg muscle power quality was calculated as power/thigh FFM (W/kg).

Time to complete a walking course (s) was determined from the better of two attempts of usual walking pace over 6 meters. The walking attempts were completed consecutively without a rest between attempts. Finally, the time to complete a five-chair-stand test without using the arms was measured (16). Each participant was asked to rise from a standard chair once without using his arms to stand. If the participant was unable to do this, he was considered unable to complete a single chair stand. If he was able to rise one time successfully, he was then asked to rise from a chair five times without using his arms; time to complete the five chair stands was recorded.

Table 1. Baseline Characteristics of White Health, Aging and Body Composition Study Participants by Alpha-Actinin-3 (ACTN3) R577X Genotype Group (N = 1367)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>RR (n = 234)</th>
<th>RX (n = 348)</th>
<th>XX (n = 144)</th>
<th>p Value</th>
<th>RR (n = 186)</th>
<th>RX (n = 330)</th>
<th>XX (n = 125)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>73.7 (3.0)</td>
<td>73.9 (2.7)</td>
<td>74.2 (3.0)</td>
<td>.228</td>
<td>73.6 (2.6)</td>
<td>73.6 (2.8)</td>
<td>73.4 (3.1)</td>
<td>.909</td>
</tr>
<tr>
<td>Height, cm</td>
<td>174.1 (6.4)</td>
<td>173.5 (6.7)</td>
<td>173.6 (5.7)</td>
<td>.524</td>
<td>159.6 (6.3)</td>
<td>159.4 (5.5)</td>
<td>159.0 (6.1)</td>
<td>.681</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>82.3 (12.9)</td>
<td>81.1 (12.2)</td>
<td>81.9 (12.7)</td>
<td>.512</td>
<td>66.4 (12.9)</td>
<td>65.8 (11.3)</td>
<td>66.5 (12.0)</td>
<td>.787</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>27.1 (3.9)</td>
<td>26.9 (3.6)</td>
<td>27.1 (3.7)</td>
<td>.762</td>
<td>26.0 (4.9)</td>
<td>25.9 (4.3)</td>
<td>26.3 (4.5)</td>
<td>.693</td>
</tr>
<tr>
<td>Fat-free mass, kg</td>
<td>58.3 (7.3)</td>
<td>57.1 (6.8)</td>
<td>58.3 (7.2)</td>
<td>.084</td>
<td>40.2 (5.4)</td>
<td>39.5 (5.1)</td>
<td>40.0 (5.2)</td>
<td>.289</td>
</tr>
<tr>
<td>Fat mass, kg</td>
<td>24.0 (7.0)</td>
<td>23.9 (6.8)</td>
<td>23.5 (6.7)</td>
<td>.824</td>
<td>26.3 (8.5)</td>
<td>26.3 (7.3)</td>
<td>26.7 (7.6)</td>
<td>.880</td>
</tr>
<tr>
<td>Body fat, %</td>
<td>27.8 (4.6)</td>
<td>29.1 (4.9)</td>
<td>28.2 (4.5)</td>
<td>.226</td>
<td>38.7 (6.0)</td>
<td>39.2 (5.4)</td>
<td>39.3 (5.4)</td>
<td>.500</td>
</tr>
<tr>
<td>Physical activity, kcal/wk</td>
<td>7650 (6416)</td>
<td>6290 (5165)</td>
<td>7021 (5632)</td>
<td>.020</td>
<td>5722 (4233)</td>
<td>5743 (4271)</td>
<td>6102 (4544)</td>
<td>.693</td>
</tr>
</tbody>
</table>

Note: Values are means (standard deviation).

Genotyping

Genotyping for the ACTN3 R577X variant (dbSNP rs1815739) was done using a TaqMan allelic discrimination assay that used the 5’ nucleotide activity of Taq polymerase to detect a fluorescent reporter signal generated during polymerase chain reactions (PCRs) as previously described (4). Both alleles were detected simultaneously using allele-specific oligonucleotides labeled with different fluorophores, and R577X genotype was determined by the ratio of the two fluorophores used.

Potential Confounders and Effect Modifiers

In both cohorts, potential confounders and effect modifiers of the association between ACTN3 R577X genotype group and muscle function, physical performance, and incident PLL were assessed. These included baseline age, body mass index, smoking status, alcohol use, comorbidity, level of physical activity, study site, baseline values, and change in body weight (for longitudinal analyses). In addition, cross-sectional data were adjusted for medications (ace inhibitor, diuretic, androgen, and anti-inflammatory use) that could have had an influence on muscle phenotypes.

Smoking status was defined as never, previous, or current smoking at baseline. In the Health ABC cohort, alcohol use was defined as self-report at baseline of less than one drink per week, one to seven drinks per week, more than one drink per day, or none. In the MrOS cohort, alcohol use was defined by self-report at baseline of none, less than one drink per week, one to seven drinks per week, more than one drink per week, or two or more drinks per week. Comorbidity in the Health ABC cohort was examined by summing the total of 11 conditions at baseline, assessed by self-report and validated with medication review, and grouping those with none, one, two, or three or more conditions. Comorbidity in the MrOS cohort was determined by summing eight conditions at baseline with a grouping that was the same as in the Health ABC Study. In the Health ABC cohort, physical activity was defined using the caloric expenditure (25) in the past week at baseline for self-reported walking, climbing stairs, and exercise. In the MrOS cohort, physical activity was assessed using the Physical Activity Scale for the Elderly (PASE) (26). Additional physical activity questions were used to assess walking for exercise, inactive time, and volunteer or paid employment (16).
Table 2. Baseline Characteristics of White Osteoporotic Fractures in Men Study Participants by Alpha-ACTN3 (ACTN3) R577X Genotype Group (N = 1152)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>RR (N = 361)</th>
<th>RX (N = 240)</th>
<th>XX (N = 361)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>73.4 (5.8)</td>
<td>73.9 (5.7)</td>
<td>73.9 (5.5)</td>
<td>.410</td>
</tr>
<tr>
<td>Height, cm</td>
<td>173.5 (6.6)</td>
<td>173.0 (6.6)</td>
<td>173.6 (6.8)</td>
<td>.306</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>85.1 (14.6)</td>
<td>83.4 (12.7)</td>
<td>85.8 (13.2)</td>
<td>.039</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>28.2 (4.4)</td>
<td>27.9 (3.9)</td>
<td>28.5 (4.1)</td>
<td>.142</td>
</tr>
<tr>
<td>Fat-free mass, kg</td>
<td>60.5 (7.8)</td>
<td>59.9 (7.3)</td>
<td>61.1 (7.4)</td>
<td>.109</td>
</tr>
<tr>
<td>Body fat, %</td>
<td>26.9 (5.5)</td>
<td>26.4 (5.2)</td>
<td>26.9 (5.2)</td>
<td>.334</td>
</tr>
<tr>
<td>Physical activity (PASE) score</td>
<td>151.5 (63.9)</td>
<td>149.1 (62.3)</td>
<td>154.2 (62.3)</td>
<td>.563</td>
</tr>
</tbody>
</table>

Notes: Values are means (standard deviation). PASE = Physical Activity Scale for the Elderly.

Data Analysis

Descriptive statistics (mean [standard deviation]) were used to describe demographic and key clinical characteristics of the study population at baseline by ACTN3 genotype groups. Because several reports have shown that the effect of this polymorphism may be sex specific (4,5), men and women were analyzed separately. For longitudinal change analyses, baseline values were subtracted from follow-up values (4 year [MrOS] or 5 year [Health ABC]). Analysis of variance (ANOVA) was used to test for overall group differences and the differences between R-homozygotes and heterozygotes versus X-homozygotes in the distribution of continuous variables. The chi-square test was used to test for differences in the distribution of categorical variables. Adjusted least-square mean values by ACTN3 R577X genotype for muscle and physical performance phenotypes were estimated in both cohorts using analysis of covariance (ANCOVA). For incident PLL, Cox’s proportional hazards models were used while adjusting for potential confounders. Statistical significance was considered when the alpha was < 0.05. Analyses were conducted with SAS (version 9.1; SAS Institute Inc., Cary, NC).

RESULTS

Genotype Results

In the Health ABC cohort, genotype distribution for all participants with genotype data fit Hardy–Weinberg equilibrium with a distribution of RR = 473 (30%), RX = 777 (50%), XX = 301 (19%) (χ² = 0.32, df = 1, p = .570). In the MrOS cohort, the genotype distribution of the white participants met the expectations of Hardy–Weinberg equilibrium with a genotype distribution of RR = 361 (31%), RX = 551 (48%), XX = 240 (21%) (χ² = 1.23, df = 1, p = .540).

Characteristics of the 1367 Health ABC participants by sex and ACTN3 genotype group are shown in Table 1. In men, there was a significant difference between the three genotype groups only for physical activity (p = .020). There were no differences between any of the other characteristics in men, and there were no differences in participant characteristics by ACTN3 genotype group in women. Table 2 shows the baseline participant characteristics of the 1152 men from the MrOS cohort by ACTN3 genotype group. There was an overall between-genotype difference only in weight, with heterozygotes weighing slightly less than R- and X-homozygotes (p = .039). There were no other baseline characteristic differences between genotype groups in the MrOS cohort.

Table 3 shows the adjusted baseline differences in muscle and performance phenotypes by sex and ACTN3 genotype in the Health ABC cohort. There were no adjusted differences between genotype groups in men or women for these phenotypes (all p > .05). Table 4 shows the mean baseline differences between ACTN3 genotype groups for muscle and performance phenotypes at baseline in the MrOS cohort. There were no adjusted differences between genotype groups for any of the phenotypes.

Five-year changes in muscle and performance phenotypes by sex and ACTN3 genotype in the Health ABC cohort are shown in Table 5. In men, there was a significant difference across genotypes (p = .030) for adjusted increases in

Table 3. Baseline Differences in Grip Strength, Knee Extensor Torque, Thigh Muscle Area, Muscle Quality, SPPB Score, 400-Meter Walk Time, and Ability to Complete 400-Meter Walk Measures in White Health, Aging and Body Composition Study Participants (N = 1367) by Alpha-ACTN3 (ACTN3) R577X Genotype Group and Sex

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Men (N = 726)</th>
<th>Women (N = 641)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RR (N = 234)</td>
<td>RX (N = 348)</td>
</tr>
<tr>
<td>Average quadriceps torque, N-m*</td>
<td>128 ± 4</td>
<td>129 ± 4</td>
</tr>
<tr>
<td>Midthigh muscle area, cm²³</td>
<td>127 ± 2</td>
<td>125 ± 2</td>
</tr>
<tr>
<td>Muscle strength, N-m/cm²³</td>
<td>2.15 ± 0.05</td>
<td>2.14 ± 0.05</td>
</tr>
<tr>
<td>Grip strength, kg</td>
<td>37 ± 0.9</td>
<td>37 ± 0.9</td>
</tr>
<tr>
<td>SPPB score</td>
<td>10.3 ± 0.2</td>
<td>10.2 ± 0.2</td>
</tr>
<tr>
<td>400-meter walk, s</td>
<td>325 ± 6</td>
<td>322 ± 6</td>
</tr>
<tr>
<td>Inability to complete 400-meter walk, n (%)</td>
<td>26 (11.1)</td>
<td>48 (13.8)</td>
</tr>
</tbody>
</table>

Notes: Values are least square means ± standard error of the mean adjusted for baseline age, body mass index, alcohol use, comorbidity, smoking, study site, physical activity, ace inhibitor use, diuretic use, and antiinflammatory medication use.

*Isokinetic torque at an angular velocity of 60 degrees/s.
1Midthigh cross-sectional area measured from computed tomography.
2Peak torque/quadiceps muscle area.
SPPB = short physical performance battery (sum of three subscores).
Four-year changes in muscle and performance phenotypes by ACTN3 genotype in the MrOS cohort are shown in Table 7. There were no differences between genotype groups for age-related adjusted changes in these phenotypes.

### DISCUSSION

To our knowledge, this is the first study to examine the influence of the ACTN3 R577X polymorphism on a diverse array of important skeletal muscle and performance phenotypes in two large, well-characterized cohorts of older adults. Moreover, this study was also able to determine the influence of the ACTN3 R577X genotype on changes in these key phenotypes with at least 4 years of aging. Although the ACTN3 R577X polymorphism does not appear to influence baseline skeletal muscle phenotypes, our results indicate that the ACTN3 X-homozygous genotype may play a small role in hastening the decline in 400-meter walk time in men with age and may increase risk of functional limitation in women. Our findings do not support an extensive influence of ACTN3 genotype on typical muscle phenotypes and provide only weak support for a potential age-related influence on the decline of physical function in late life.

It is unclear why there were only differences in 400-meter walk time in men by ACTN3 genotype. Previous reports have shown that the ACTN3 genotype may be sex specific, with greater effects observed on skeletal muscle–related phenotypes in women (4,5). The results of the present investigation showing a difference in incident functional limitation in only women by ACTN3 genotype is consistent with the findings of those previous investigations. However, there were no other associations observed in women, which suggests that the influence of the ACTN3 genotype does not have a strong effect on these phenotypes. One possible explanation for the unexpected 400-meter-walk finding in...
muscle power quality, $W_{\text{k}}$ (5, 6).5

Five-chair-stand time, s 1.0

X-homozygotes showing greater increases in walk time.

However, the previously observed associations between the performance in younger cohorts and in athletes (5, 7, 8, 27) are surprising given the recent findings that the genotype and baseline skeletal muscle traits is somewhat due to chance. Moreover, the 400-meter-walk results by men could be that there were a host of phenotypes examined, which could have led to a spurious association due to chance. Moreover, the 400-meter-walk results by genotype, although not significant, were in the opposite direction in women, and there was not a similar phenotype in the MrOS cohort to confirm the results in men. However, the association between changes in 400-meter walk time and $ACTN3$ genotype was in the expected direction, with X-homozygotes showing greater increases in walk time.

The lack of an association between the $ACTN3$ R577X genotype and baseline skeletal muscle traits is somewhat surprising given the recent findings that the $ACTN3$ R577X genotype has a significant influence on physical and muscle performance in younger cohorts and in athletes (5, 7, 8, 27) and based on the large sample size of the current study. However, the previously observed associations between the $ACTN3$ polymorphism and skeletal muscle-related phenotypes have been observed primarily in the presence of interventions such as exercise training (4, 9). Although some cross-sectional reports also indicate an association between athletic performance and the $ACTN3$ genotype, these participants were highly trained athletes and may not be representative of the general population (5–8, 28). Nevertheless, our findings support those from smaller cross-sectional cohorts that the $ACTN3$ polymorphism does not explain a significant proportion of the variability in muscle phenotypes in older adults (9, 10).

The considerable changes in skeletal muscle structure and physiology that occur with age may be at least a partial explanation for the lack of a significant relationship between skeletal muscle phenotypes and the $ACTN3$ R577X polymorphism. For example, there are many structural changes in skeletal muscle that occur with age, including increased fat and connective tissue within skeletal muscle, loss of motor units, and the loss of type II muscle fibers (29). This loss of type II muscle fibers may be particularly important with regard to the influence of the $ACTN3$ R577X SNP in elderly persons, as $ACTN-3$ is expressed in this fiber type only. A significant loss of type II fibers would have a much greater influence on muscle function than would merely a lack of $ACTN-3$ expression alone, and would amplify the importance of type I skeletal muscle fibers in physical functioning. In addition, recent research has found that type IIX fiber number is lower in X-homozygotes than in R-homozygotes (14). Other recent findings (30) in $ACTN3$ knockout mice indicate that metabolic and structural properties of type II skeletal muscle fibers change to favor endurance performance. These data suggest a mechanistic pathway by which the $ACTN3$ genotype may influence muscle performance. However, these findings have not been confirmed in older adults. Further exploration of the functional contributions of the $ACTN-3$ protein would help to clarify whether this polymorphism should continue to be investigated with regard to its association with physical performance.

Although there are numerous strengths of the current investigation, there are several potential limitations that should be addressed. First, the MrOS cohort should not be considered a true validation cohort for the Health ABC cohort based on current standards (31), as not all of the phenotypes were the same or measured in the same way and the MrOS sample did not include women. However, a

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### Table 6. Incident Persistent Lower Extremity Limitation (PLL) in White Health, Aging and Body Composition Study Participants by $Sex$ and $Alpha-Actinin-3 (ACTN3)$ $R577X$ Genotype ($N = 1367$)

<table>
<thead>
<tr>
<th>Incident PLL</th>
<th>Number of Events</th>
<th>Number of Events/100 Person-Years</th>
<th>Unadjusted HR (95% CI)</th>
<th>Adjusted HR* (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Men ($n = 726$)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RR</td>
<td>234</td>
<td>70</td>
<td>8.2</td>
<td>1.02 (0.70–1.50)</td>
</tr>
<tr>
<td>RX</td>
<td>348</td>
<td>93</td>
<td>7.2</td>
<td>0.90 (0.63–1.30)</td>
</tr>
<tr>
<td>XX</td>
<td>144</td>
<td>42</td>
<td>8.1</td>
<td>1.00</td>
</tr>
<tr>
<td><strong>Women ($n = 641$)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RR</td>
<td>186</td>
<td>57</td>
<td>8.5</td>
<td>0.64 (0.44–0.93)</td>
</tr>
<tr>
<td>RX</td>
<td>330</td>
<td>126</td>
<td>11.0</td>
<td>0.82 (0.60–1.21)</td>
</tr>
<tr>
<td>XX</td>
<td>125</td>
<td>55</td>
<td>13.5</td>
<td>1.00</td>
</tr>
</tbody>
</table>

**Notes:** Data presented are raw and adjusted hazard ratios (HR) and 95% confidence intervals (CI).

*Data adjusted for baseline age, body mass index, comorbidity, smoking status, alcohol use, study site, and physical activity.

### Table 7. Four-Year Changes in Grip Strength, Leg Muscle Power, Thigh Fat-Free Mass, Muscle Quality, and Physical Functioning Measures in White Osteoporotic Fractures in Men Study Participants by $Alpha-Actinin-3 (ACTN3)$ $R577X$ Genotype Group

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>RR ($N = 274$)*</th>
<th>RX ($N = 392$)†</th>
<th>XX ($N = 185$)‡</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak power, $W$</td>
<td>$-37 \pm 4$</td>
<td>$-45 \pm 4$</td>
<td>$-41 \pm 54$</td>
<td>.181</td>
</tr>
<tr>
<td>Thigh muscle FFM, kg</td>
<td>$-0.6 \pm 0.1$</td>
<td>$-0.7 \pm 0.1$</td>
<td>$-0.6 \pm 0.1$</td>
<td>.573</td>
</tr>
<tr>
<td>Muscle power quality, $W$</td>
<td>$-1.7 \pm 0.2$</td>
<td>$-2.1 \pm 0.2$</td>
<td>$-1.8 \pm 0.2$</td>
<td>.303</td>
</tr>
<tr>
<td>Grip strength, kg</td>
<td>$-2.9 \pm 0.4$</td>
<td>$-2.8 \pm 0.4$</td>
<td>$-1.7 \pm 0.5$</td>
<td>.092</td>
</tr>
<tr>
<td>Five-chair-stand time, s</td>
<td>$1.0 \pm 0.3$</td>
<td>$1.1 \pm 0.2$</td>
<td>$1.2 \pm 0.3$</td>
<td>.798</td>
</tr>
<tr>
<td>6-Meter walk, m/s $\times 10^{-1}$</td>
<td>$-1.4 \pm 0.1$</td>
<td>$-1.4 \pm 0.1$</td>
<td>$-1.6 \pm 0.2$</td>
<td>.568</td>
</tr>
</tbody>
</table>

**Notes:** Values are LS means ± standard error of the mean adjusted for baseline age, body mass index, comorbidity, alcohol use, smoking status, physical activity, study site, baseline values, and body weight change.

*Missing data points: 100 for peak power, 103 for muscle power quality, 12 for grip strength, 39 for five chair stands, and 7 for 6-meter walk.
†Missing data points: 172 for peak power, 173 for muscle power quality, 22 for grip strength, 52 for five chair stands, and 18 for 6-meter walk.
‡Missing data points: 71 for peak power, 72 for muscle power quality, 13 for grip strength, 58 for five chair stands, and 11 for 6-meter walk.
$^3$Peak power of both legs using the Nottingham Power Rig.
$^4$Peak muscle power/thigh FFM.
FFM = fat-free mass.
meaningful genetic influence should be strong enough to
capture closely related phenotypes. Second, there was some
loss to follow-up in both cohorts that may have biased our
results. However, the X-homozygote genotype frequency in
both the baseline and follow-up subsamples were ~20%,
suggesting that this loss to follow-up was unlikely related to
ACTN3 genotype. Third, skeletal muscle and performance
phenotypes are complex phenotypes that are influenced by
environmental/lifestyle factors and numerous genetic fac-
tors. We did not consider the entire complement of can-
didate gene polymorphisms in the present analysis. There are
likely complex Gene × Gene and Gene × Environment
interactions not yet explored that could help to explain inter-
individual variability in muscle phenotypes. Finally, al-
though the length of follow-up observed in the present
investigation has been shown to be long enough to observe
significant phenotype changes in older adults (21,32,33),
a longer follow-up interval may be necessary.

Summary

Although the ACTN3 R577X polymorphism was associ-
ated with two longitudinal measures of physical functioning,
it does not appear to play a consistent or role in the
age-related declines in physical functioning in older adults.
Additionally, the ACTN3 R577X polymorphism does not
appear to play a significant role in baseline skeletal muscle
phenotypes in the general population of older adults.
Continued examination of this candidate gene is warranted for
intervention studies, as this gene has been shown to
explain a greater proportion of trait variance in response to
interventions such as resistance training (4,9). Additional
studies with a longer follow-up in older adults and with
measures that are more specific to type II muscle fiber
functioning, such as peak movement velocity, may also help
to determine if this polymorphism plays a significant role in
muscle function in the aging process.

ACKNOWLEDGMENTS

The Health ABC study was supported by research contracts #1AG-6-
2101, #1AG-6-2103, and #1AG-6-2106 from the National Institute on
Aging (NIA). This study was supported by intramural funding. The following institutes provide support: the
National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS), the NIA, the National Center for Research Resources (NCRR),
and NIH Roadmap for Medical Research under the following grant
numbers: U01 AR45580, U01 AR45614, U01 AR45632, U01 AR45647,
U01 AR45654, U01 AR45583, U01 AG18197, U01 AG027810, and UL1
RR024140. Additional support was also provided by NIAMS grants
R01-AR051124, R01-AG021024, AG-027971, the NIA Aging Training
Grant, and a grant from the Department of Epidemiology, University of
Pittsburgh.

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REFERENCES

1. Arden NK, Spector TD. Genetic influences on muscle strength, lean

2. Huygens W, Thomis MA, Peeters MW, Vlietinck RF, Beunen GP.
Determinants and upper-limit heritabilities of skeletal muscle mass and

effect of strength training on skeletal muscle strength in older adults..

4. Clarkson PM, Devaney JM, Gordish-Dressman H, et al. ACTN3
polyorphism associated with increases in muscle strength in response to

5. Yang N, MacArthur DG, Guibin JP, et al. ACTN3 genotype is

6. Santiago C, Gonzalez-Freire M, Serra-Russo L, et al. ACTN3 genotype in

7. Papadimitriou ID, Papadopoulous C, Kouvassi A, Triantaphylidis C.
The ACTN3 gene in elite Greek track and field athletes. Int J Sports

ACTN3 R577X nonsense allele is under-represented in elite-level

(ACTN3) R577X polymorphism influences knee extensor peak power
response to strength training in older men and women. J Gerontol A

10. San Juan AF, Gomez-Gallego F, Canete S, Santiago C, Perez M,
Lucia A. Does complete deficiency of muscle alpha actin 3 alter

AH. A common nonsense mutation results in alpha-actinin-3 deficiency in

(ACTN3) occurs in different forms of muscular dystrophy. Neuropa-

the actin-binding proteins, a-actin-2 and -3, in different species:
implications for the evolution of functional redundancy. Hum Mol

is associated with fiber type distribution. Physiol Genomics. 2007;32:
58–63.

quality in a well-functioning cohort of older adults: the Health,
330.

characteristics of the osteoporotic fractures in men (MrOS) study—
a large observational study of the determinants of fracture in older men.

17. Blank JB, Cawthon PM, Carrion-Petersen ML, et al. Overview of
recruitment for the osteoporotic fractures in men study (MrOS).

18. Visscher M, Krutchevsky SB, Goodpaster BH, et al. Leg muscle mass and
composition in relation to lower extremity performance in men and
women aged 70 to 79: the Health, Aging and Body Composition study.

19. Guralnik JM, Ferrucci L, Simonsick EM, Salive ME, Wallace RB.
Lower-extremity function in persons over the age of 70 years as a
561.

performance battery assessing lower extremity function: association with
self-reported disability and prediction of mortality and nursing home

distance corridor walk performance with mortality, cardiovascular
disease, mobility limitation, and disability. JAMA. 2006;295:
2018–2026.

22. Harkonen R, Harju R, Alaranta H. Accuracy of the Jamar dynamom-

23. Bassey EJ, Short AH. A new method for measuring power output in
a single leg extension: feasibility, reliability and validity. Eur J Appl


Received March 18, 2008
Accepted July 4, 2008
Decision Editor: Luigi Ferrucci, MD, PhD