The Functional ACTN3 577X Variant Increases the Risk of Falling in Older Females: Results From Two Large Independent Cohort Studies

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Background. Falls among elderly people is a major issue in public health, causing debilitating outcomes including fracture. The identification of genetic risk factors for falling may provide a strategy for effectively targeting falls prevention programs. We investigated whether a common functional variant of skeletal muscle α-actinin-3 (ACTN3 p. R577X) previously associated with impairments in muscle strength, power, and physical functioning represents a risk factor for falls.

Methods. Case–control analysis was conducted using two large cohorts of Caucasian postmenopausal women—the North of Scotland Osteoporosis Study (n = 1,245) and the Aberdeen Prospective Osteoporosis Screening Study (n = 2,918)—for whom self-reported falls status and DNA samples were available. Cross-sectional analysis of fallers versus nonfallers at baseline and follow-up was performed. In addition, individuals who reported having fallen at more than one timepoint (recurrent fallers) were compared with those who reported not falling at any timepoint.

Results. Association between R577X genotype and falls was identified and validated. Carriage of 577X (one or two copies) was significantly associated with a 33% (10%–61%) increased risk of falling, with the effect apparent at both baseline and follow-up assessments (meta-analysis p = .003 and p = .02, respectively). No significant effect on recurrent falls was observed.

Conclusion. This study reports for the first time that the functional ACTN3 R577X genotype represents a genetic risk factor for falling in older females.

Key Words: Falls—ACTN3—Genetic association—Skeletal muscle.

Received May 20, 2010; Accepted August 24, 2010

Decision Editor: Luigi Ferrucci, MD, PhD
genotype is associated with reduced knee extensor torque (15), lower thigh muscle cross-sectional area (16), and increased risk of lower extremity limitation (17). Collectively, these data suggest that ACTN3 deficiency is detrimental to skeletal muscle function. Because impaired physical functioning and muscular weakness are major risk factors for falling in elderly people (18), we therefore hypothesized that R577X genotype may influence falls risk among older females.

**METHODS**

**Participants**

Data and samples from two large, Scottish population-based cohorts of postmenopausal women characterized for falls status were accessed, the North of Scotland Osteoporosis Study (NOSOS) (19) and the Aberdeen Prospective Osteoporosis Screening Study (APOSS) (20). Participants self-reported falls at multiple timepoints using the question, “Have you fallen in the last year?” Information on age at assessment, height, weight, current medications, concomitant medical conditions (CMC), smoking status, alcohol intake, and socioeconomic status were also available. Details are given in Supplementary Methods.

For baseline and follow-up assessments, participants were classified as “fallers” (reported falling in the previous year) or “nonfallers” (reported not having fallen in the previous year). Individuals were further classified using their responses at follow-up assessments according to whether they were “recurrent fallers” (reported having fallen in the previous year at more than one assessed timepoint) or “never fell” (reported they did not fall in the previous year at all assessed timepoints). For analysis, individuals were also categorized according to whether or not they used medications that may affect mobility or balance (diuretics, antiepileptics, or other drugs affecting the central nervous system) and whether or not they self-reported a CMC that affect balance or mobility (visual problems, previous stroke, osteoarthritis, rheumatoid arthritis, multiple sclerosis, or Parkinson’s disease). Smoking status was recorded as current, past, or never. Participants’ socioeconomic status was determined from postal code deprivation categories, with 1 being low deprivation and 7 being high deprivation (21). Alcohol intake in grams per day in the week prior to assessment was calculated from Food Frequency Questionnaire responses (22). Because alcohol intake was not normally distributed, it was coded as tertiles within each cohort.

This study was approved by Grampian Research Ethics Committee (01/0161) and North of Scotland Research Ethics Committee (07/S0802/121, 10/S0801/4).

**Study Power**

Prospective power to detect association with baseline falls was calculated separately for NOSOS and APOSS using Quanto version 1.2.3 (23). For each study, baseline falls prevalence was calculated from the data. ACTN3 genotype frequencies had been reported previously (7). Under an additive model, it was estimated that NOSOS (n = 357 fallers, 2.5 controls per case) had 82% power and APOSS (n = 638 fallers, 3.6 controls per case) had 98% power to detect an association with baseline falls for genotype risks with an odds ratio (OR) of 1.3 at the 5% significance level.

**Genotyping**

APOSS DNA sample extraction has been described previously (20). For NOSOS, genomic DNA was extracted from peripheral venous blood samples by magnetic bead separation using GeneCatcher gDNA Blood Kits (Invitrogen Ltd, Paisley, UK) and quantitated using the QuantIT PicoGreen dsDNA Quantitation Kit (Invitrogen Ltd).

NOSOS DNA samples were genotyped for R577X using a previously reported polymerase chain reaction–restriction fragment polymorphism assay (24). One sample for each genotype was sequenced by the University of Dundee Sequencing Service (Dundee, UK) to confirm genotype and used as positive controls in genotyping assays. Genotypes were called by two independent observers and discrepancies re-genotyped. Repeat genotyping of 3% samples was performed to assess error rate.

APOSS DNA samples were genotyped using hybridization probe dissociation curves on a LightCycler480 instrument (Roche, Burgess Hill, UK). Details are given in Supplementary Methods. Genotyping was repeated on 3% samples to assess error rate. NOSOS repeats genotyped by polymerase chain reaction–restriction fragment polymorphism assay were also genotyped using hybridization probes to assess concordance between methodologies.

**Statistical Analysis**

Statistical analyses were performed using SPSS17 (SPSS Inc., Somers, NY) unless stated otherwise. Hardy–Weinberg equilibrium testing was performed using PLINK v1.03 (25).

Data from NOSOS and APOSS were analyzed separately with respect to baseline falls and recurrent falls status. Demographic differences were examined using analysis of variance for continuous variables or chi-square analysis for categorical variables. Genotypes were coded 1 = RR, 2 = RX, and 3 = XX for examination of independent genotype and additive effects. Genotype groups were collapsed to test dominant (any X versus RR) and recessive (XX versus any R) effects. Associations with falls status were examined by logistic regression, with correction for confounders. Confounders were included if different at p ≤ .05 between cohorts, between fallers and nonfallers (irrespective of genotype), or by genotype (irrespective of falls status). Results are expressed as OR and 95% confidence interval (CI) with reference to nonfallers. Significant associations with baseline falls were then assessed at follow-up using the
Table 1. Baseline Demographics for Individuals With Falls Status and Genotype Data Available

<table>
<thead>
<tr>
<th>Cohort</th>
<th>NOSOS</th>
<th>APOSS</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>1,245</td>
<td>2,918</td>
<td>—</td>
</tr>
<tr>
<td>Age (y)</td>
<td>69.6 ± 5.5</td>
<td>54.8 ± 2.2</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>158 ± 6</td>
<td>160 ± 6</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>67.7 ± 12.3</td>
<td>68.5 ± 12.8</td>
<td>.07</td>
</tr>
<tr>
<td>Smoking*</td>
<td>58:31:11</td>
<td>50:32:19</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Alcohol intake†</td>
<td>36:31:33</td>
<td>34:33:33</td>
<td>.3</td>
</tr>
<tr>
<td>Medications</td>
<td>33%</td>
<td>11%</td>
<td>.001</td>
</tr>
<tr>
<td>CMC</td>
<td>46%</td>
<td>9%</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>

Notes: Mean ± SD shown for continuous variables with corresponding analysis of variance p values for differences between cohorts. Percentage with outcome within each cohort is shown for binary outcomes and corresponding p values calculated from Pearson chi-square with 1 df. APOSS = Aberdeen Prospective Osteoporosis Screening Study; CMC = concomitant medical condition; NOSOS = North of Scotland Osteoporosis Study.

* Percentage of never, past, and present smokers, respectively; p value calculated from Pearson chi-square with 2 df.
† Percentage of low, mid, and high alcohol intake, respectively; p value calculated from Pearson chi-square with 2 df.
‡ Percentage within national deprivation categories 1–6, respectively; p value calculated from Pearson chi-square with 5 df.

appropriate genetic model to assess within-cohort reproducibility of effect.

An estimate of the overall effect of genotype on falls status across cohorts was determined by random effects meta-analysis using Stata v11.0 (StataCorp, College Station, TX) metan command (26) with corrected ORs and 95% CI obtained from logistic regression and expressed as pooled OR and 95% CI.

RESULTS

For NOSOS, 1,245 DNA samples (95%) were successfully genotyped and 2,918 APOSS DNA samples (94%) were genotyped. Concordance for repeat genotyping and between methods was 100%. Genotypes were in Hardy–Weinberg equilibrium within each cohort.

Compared with the APOSS population, the NOSOS population was significantly older, shorter, and had more CMC and medication use (p < .001; Table 1). APOSS participants were more likely to have smoked and lived in less deprived areas (p < .001; Table 1). Falls prevalence at baseline was 29% in NOSOS and 22% in APOSS; 18% NOSOS and 5% APOSS were recurrent fallers. For both cohorts, there were significant effects of baseline age, weight, alcohol intake, medications, and CMC on falls status (p ≤ .05; Supplementary Table).

Genotype frequencies did not differ significantly between cohorts (p = .2). There were no significant differences in baseline age, weight, smoking status, alcohol intake, socioeconomic status, medications, and CMC between genotype groups for either cohort nor for height in APOSS (Table 2).

In NOSOS, women with RX were shorter by 1 cm compared with RR and XX (p = .005). Genotype distributions with each cohort by falls status are shown in Table 3.

Table 2. Baseline Demographics by Genotype for Each Cohort

<table>
<thead>
<tr>
<th>Genotype</th>
<th>RR</th>
<th>RX</th>
<th>XX</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>NOSOS</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N (%)</td>
<td>359 (29)</td>
<td>648 (52)</td>
<td>238 (19)</td>
<td>—</td>
</tr>
<tr>
<td>Age (y)</td>
<td>69.4 ± 5.4</td>
<td>69.8 ± 5.5</td>
<td>69.6 ± 5.6</td>
<td>.5</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>159 ± 6</td>
<td>158 ± 6</td>
<td>159 ± 6</td>
<td>.005</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>67.9 ± 12.8</td>
<td>67.3 ± 12.4</td>
<td>68.6 ± 11.3</td>
<td>.4</td>
</tr>
<tr>
<td>Alcohol intake†</td>
<td>33:33:34</td>
<td>37:30:33</td>
<td>41:28:31</td>
<td>.6</td>
</tr>
<tr>
<td>Medications</td>
<td>36%</td>
<td>31%</td>
<td>31%</td>
<td>.3</td>
</tr>
<tr>
<td>CMC</td>
<td>44%</td>
<td>42%</td>
<td>45%</td>
<td>.8</td>
</tr>
</tbody>
</table>

Notes: Mean ± SD shown for continuous variables, with corresponding analysis of variance p values for differences between genotype groups. For binary variables, percentage with outcome within each genotype group is shown; corresponding p values were calculated from Pearson chi-square with 2 df. APOSS = Aberdeen Prospective Osteoporosis Screening Study; CMC = concomitant medical condition; NOSOS = North of Scotland Osteoporosis Study.

* Percentage of never, past, and present smokers, respectively; p value calculated from Pearson chi-square with 4 df.
† Percentage of low, mid, and high alcohol intake, respectively; p value calculated from Pearson chi-square with 4 df.
‡ Percentage within national deprivation categories 1–6, respectively; p value calculated from Pearson chi-square with 10 df.

Because of the significant differences in demographics between groups according to falls status, cohort and genotype described above, logistic regression analyses were corrected for age, height, weight, smoking status, alcohol

Table 3. R577X Genotype Distribution According to Falls Status

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Nonfaller</th>
<th>Faller</th>
<th>Nonfaller</th>
<th>Faller</th>
</tr>
</thead>
<tbody>
<tr>
<td>NOSOS</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R/R</td>
<td>262 (30)</td>
<td>97 (27)</td>
<td>699 (31)</td>
<td>184 (29)</td>
</tr>
<tr>
<td>R/X</td>
<td>455 (51)</td>
<td>193 (54)</td>
<td>1.115 (49)</td>
<td>319 (50)</td>
</tr>
<tr>
<td>X/X</td>
<td>171 (19)</td>
<td>67 (19)</td>
<td>466 (20)</td>
<td>135 (21)</td>
</tr>
<tr>
<td>Total</td>
<td>888</td>
<td>357</td>
<td>2,280</td>
<td>638</td>
</tr>
</tbody>
</table>

Notes: Distribution of genotypes (number and percentage within group) according to falls status and cohort is shown for cross-sectional baseline falls assessment and for assessment of recurrent falls across all timepoints (longitudinal). In all cases, RR genotype frequency is lower and RX + XX frequency is higher among fallers compared with corresponding nonfallers. APOSS = Aberdeen Prospective Osteoporosis Screening Study; NOSOS = North of Scotland Osteoporosis Study.
intake, socioeconomic status, medications, and CMC. Meta-analysis indicated no significant between-cohort heterogeneity ($P_{\text{heterogeneity}} > .5$, all $P = 0$).

Baseline falls were associated with presence of one or two copies of 577X (Table 4). Under a dominant model, this result was significant in both NOSOS ($OR = 1.43$, 95% CI: 1.00–2.04, $p = 0.049$) and APOSS ($OR = 1.30$, 95% CI: 1.04–1.62, $p = 0.02$) independently. The pooled OR across both cohorts was 1.33 (95% CI: 1.10–1.61, $p = 0.003$). Analysis of independent genotype effects supported the dominant model rather than an additive effect because the pooled OR for comparison of RX versus RR was the same as for XX versus RR with similar 95% CIs (RX: $OR_{\text{pooled}} = 1.33$ [1.09–1.62], $p = 0.02$; XX: $OR_{\text{pooled}} = 1.34$ [1.05–1.71], $p = .005$).

The effect of the 577X allele on falling in the previous year was also evident for the follow-up falls assessment (dominant model: $OR_{\text{pooled}} = 1.33$ [1.06–1.66], $p = .01$). In the individual cohorts, the effect sizes were similar to those at baseline but did not reach statistical significance likely due to reduced sample size (NOSOS 2004 follow-up: OR = 1.39 [0.95–2.02], $p = 0.9$, $n = 806$; APOSS 2002 follow-up: OR = 1.30 [0.98–1.71], $p = .07$, $n = 2,070$).

No significant effect of R577X genotype was observed for recurrent falls under any model ($p \geq .2$; Table 4). Sample numbers for recurrent falls analysis were considerably reduced from the baseline analyses (Table 3), and the study lacked power to detect association with recurrent falls. However, the effect sizes for recurrent falls (dominant model $OR_{\text{pooled}} = 1.24$ [0.87–1.77]) are smaller than those from the cross-sectional analyses, suggesting that R577X does not increase the risk of suffering falls at multiple timepoints over and above that observed for a single timepoint.

### Table 4. Effect of R577X on Baseline and Recurrent Falls Status

<table>
<thead>
<tr>
<th></th>
<th>NOSOS</th>
<th>APOSS</th>
<th>Pooled</th>
<th>Pooled p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline falls</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Additive</td>
<td>1.21 (.96–1.51)</td>
<td>1.15 (1.00–1.32)</td>
<td>1.17 (1.04–1.31)</td>
<td>.01</td>
</tr>
<tr>
<td>RX vs RR</td>
<td>1.43 (0.99–2.07)</td>
<td>1.29 (1.02–1.63)</td>
<td>1.33 (1.09–1.62)</td>
<td>.005</td>
</tr>
<tr>
<td>XX vs RR</td>
<td>1.43 (0.90–2.26)</td>
<td>1.31 (0.99–1.74)</td>
<td>1.34 (1.05–1.71)</td>
<td>.02</td>
</tr>
<tr>
<td>Dominant</td>
<td>1.43 (1.00–2.04)</td>
<td>1.30 (1.04–1.62)</td>
<td>1.33 (1.10–1.61)</td>
<td>.003</td>
</tr>
<tr>
<td>Recurrent falls</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Additive</td>
<td>1.04 (0.73–1.48)</td>
<td>1.16 (0.88–1.55)</td>
<td>1.11 (0.89–1.39)</td>
<td>.4</td>
</tr>
<tr>
<td>RX vs RR</td>
<td>1.29 (0.71–2.36)</td>
<td>1.22 (0.76–1.97)</td>
<td>1.25 (0.86–1.81)</td>
<td>.2</td>
</tr>
<tr>
<td>XX vs RR</td>
<td>1.05 (0.50–2.19)</td>
<td>1.34 (0.76–2.39)</td>
<td>1.22 (0.78–1.93)</td>
<td>.4</td>
</tr>
<tr>
<td>Dominant</td>
<td>1.22 (0.69–2.16)</td>
<td>1.26 (0.80–1.97)</td>
<td>1.24 (0.87–1.77)</td>
<td>.2</td>
</tr>
</tbody>
</table>

Note: OR (95% CI) for association between R577X genotype and baseline and recurrent falls under additive, genotype (RX vs RR and XX vs RR), dominant (RX + XX vs RR), and recessive (RR + RX vs XX) models for each cohort is shown, corrected for age, height, weight, medications, concomitant medical conditions, smoking status, tertile of alcohol intake, and socioeconomic status. Pooled OR (95% CI) across cohorts and associated p value are also shown. CI = confidence interval; OR = odds ratio.

### Discussion

This is the first large-scale study to examine the effect of ACTN3 R577X on falls status. We identified and validated an association between R577X and falls in two large, independent population-based cohorts of women from northeast Scotland and showed that carriage of one or two copies of the null variant of ACTN3 increases the risk of falling by 33%.

The cross-sectional association between R577X genotype and falls was observed at both baseline and follow-up assessments across both cohorts. No effect was observed for the extremes of falls status longitudinally (recurrent fallers compared with never fallers), possibly due to insufficient power from the reduced sample size. However, the effect size observed for this extreme comparison does not suggest that there is any increased risk of suffering recurrent falls over and above that seen for falling in the previous year at a single timepoint. This suggests that R577X influences the ability to maintain balance after a fall has been initiated (eg, by some external environmental factor), such that individuals with RR respond rapidly and are better able to right themselves, whereas X carriers are slower to respond and less well able to prevent the fall. It would be interesting to see if this is indeed the case using gait perturbation analysis (27).

The associations reported between R577X and muscle performance in elite athletes are robust, but the influence of this polymorphism on muscle function in the general population has yet to be fully characterized. To date, ACTN3 deficiency has been shown to have a detrimental effect on elbow flexor maximum voluntary contraction (13), 40-m sprint speed (14), and knee extensor peak torque (15,28) for various groups, with age- and sex-specific effects. However, smaller studies have produced conflicting results (29–31). It is therefore critical that future studies investigating the effect of R577X on muscle function are sufficiently powered to detect small effect sizes, and caution must be given when interpreting results from those studies employing moderately sized cohorts.

The effect of R577X on muscle function in the general population appears modest, explaining ~1.5% to 3% variability in several parameters of muscle strength and power (13–15). No comprehensive data are available for elderly populations; however, the variability appears sufficient to induce a detrimental impact on muscle function among older females that is large enough to influence falls risk. It will be interesting to investigate whether other clinical outcomes associated with muscle weakness among elderly individuals, such as fracture, frailty, disability, and sarcopenia, are also associated with R577X genotype.

In this study, we observed a detrimental influence of the X allele, with RX and XX contributing equally to increased falls risk compared with RR. This differs from other studies that commonly group data from RX + RR on the assumption of phenotypic uniformity and analyze against XX, for
which significant associations with some parameters of muscle function have been reported (15,16,32). Alternatively, additive effects have been reported for strength and power traits, with the phenotype associated with RX intermediate to those for RR and XX (13,14,28,29,33). Although the effect sizes here suggest a dominant effect on falls, we are unable to rule out similar dosage effects given the CIs around our results. There are also reports which failed to identify association between skeletal muscle phenotypes and R577X (30,31,34). The precise biologic distinction between RX and RR genotypes with regard to skeletal muscle ACTN3 expression levels, dimerization and function has yet to be fully addressed in the literature; the effect of RX on these thus requires elucidation to aid interpretation of results from genetic studies.

Future characterization of additional genetic determinants of falling in elderly populations is required to permit identification of individuals at high risk of falls. It is likely that numerous polymorphisms plus gene–gene and gene–environment interactions will contribute to falls risk, given its multifactorial nature; indeed, association between vitamin D receptor gene polymorphisms and falls has recently been reported (35,36).

In this study, falling was self-reported retrospectively. Although retrospective recall has potential inaccuracies, especially when investigating older individuals who might be prone to recall bias (37), this methodology nevertheless represents an accepted, inexpensive, and invaluable form of data collection for large-scale studies. Even in the very elderly, recalled falls in the past year have been shown to predict prospective falls (38). For both cohorts, information on a number of potential confounders was available, permitting correction of results to identify independent effects of R577X. Falls status and confounders were assessed similarly between the cohorts, and genotype frequencies were also similar, enabling joint analysis of significant effects across both cohorts. This study is adequately powered to detect small genetic effects (OR ≥ 1.3) common in multifactorial outcomes, such as those now being reported for rheumatoid arthritis, type 2 diabetes, and heart disease (39).

Because we examined only women, we are unable to comment on whether or not R577X might influence falling in older males. However, the effects of R577X on physical function impairment and muscle function reported previously were found to be significant only for females (15,17), suggesting that any role for R577X in influencing falling will perhaps be less pronounced in males than in females. No muscle function measurements were available for NOSS and APOSS participants, so we are unable to assess whether the association between R577X and falls is mediated via effects on muscle function.

We examined only the R577X SNP within ACTN3, selected on the basis of previously described associations with muscle phenotypes; it remains possible that other reported missense variants within ACTN3 not examined in this study may modulate the effect described. Other factors not examined here, such as level of physical activity or proportion of type II muscle fibers, may also alter the expressivity or effect of R577X.

In summary, we have found evidence to indicate that the functional ACTN3 R577X polymorphism is a risk factor for falling among older females. Further work is required to elucidate the underlying mechanism and to identify other potential genetic risk factors for falling.

**Funding**

This work was supported by a Nuffield Foundation Oliver Bird Rheumatism Programme PhD Studentship to R.N.J. Genotyping was funded by Generation Scotland: Genetic Health in the 21st Century (Scottish Funding Council Strategic Research Development Grant HR03006).

**Supplementary Material**

Supplementary material can be found at: http://biomedgerontology.journals.org/

**Acknowledgments**

The authors gratefully acknowledge the continued support of APOSS and NOSS participants and all those involved in sample and data collection.

**References**