Role of \textit{APOE} Genotype in Gait Decline and Disability in Aging

Joe Verghese,\textsuperscript{1,2} Roe Holtzer,\textsuperscript{1} Cuiling Wang,\textsuperscript{3} Mindy J. Katz,\textsuperscript{1} Nir Barzilai,\textsuperscript{2} and Richard B. Lipton\textsuperscript{1,3}

\textsuperscript{1}Department of Neurology,\textsuperscript{2} Department of Medicine, and\textsuperscript{3} Department of Epidemiology, Albert Einstein College of Medicine, Bronx, New York.

Address correspondence to Joe Verghese, MBBS, Division of Cognitive and Motor Aging, Department of Neurology, Albert Einstein College of Medicine, 1165 Morris Park Avenue, Bronx, NY 10461. Email: joe.verghese@einstein.yu.edu

\textbf{Objectives.} Although apolipoprotein E (\textit{APOE}) genetic variation may influence risk of gait decline and disability in aging through multiple mechanisms, a systematic examination of this relationship has been lacking. Our objective was to quantify the risk of gait decline and disability associated with the \textit{APOE} \textit{ε4} allele in aging.

\textbf{Methods.} We evaluated 627 community-dwelling adults aged 70 and older (white 67.8\%) with \textit{APOE} genotype and quantitative gait measurements participating in the Einstein Aging Study over a median follow-up of 3.0 years. Main outcomes were gait speed decline (cm/s/year) and incident disability.

\textbf{Results.} \textit{APOE} \textit{ε4} allele frequency was 24.1\%. Presence of \textit{APOE} \textit{ε4} was not significantly associated with gait speed decline overall ($p = .37$) but was associated with faster gait speed decline in older men (estimate: $-1.16, 95\%$ CI: $-2.31$ to $-0.01, p = .04$). The interaction between the \textit{ε4} allele and male sex predicted gait speed decline (estimate: $-1.70, 95\%$ CI: $-3.33$ to $-0.07, p = .04$). Presence of the \textit{APOE} \textit{ε4} allele was associated with increased risk of disability in older men (HR $3.72, 95\%$ CI: $1.44$–$9.59, p = .04$). Presence of the \textit{APOE} \textit{ε4} allele with study outcomes remained significant even after accounting for several potential confounders including vascular and cognitive status. The strength of the associations was stronger in the white subgroup.

\textbf{Conclusion.} This preliminary report suggests that the \textit{APOE} \textit{ε4} allele is associated with increased risk of gait speed decline and disability in older men.

\textbf{Key Words:} Gait—Disability—Epidemiology—Genetics.

\textbf{Received January 11, 2013; Accepted June 13, 2013}

\textbf{Decision Editor: Stephen Kritchevsky, PhD}

\textbf{Gait.} Gait speed is considered a vital sign in older adults (1,2). Although the biology of decline in gait speed with aging is only partially understood, it is influenced by aging and diseases (3). The role of heredity in gait speed decline, however, is not well established.

The apolipoprotein \textit{E} (\textit{APOE}) gene is known to influence brain disease and best known as a risk factor for Alzheimer’s disease (4) and to a lesser extent for cardiovascular disease (5). Although cognitive and vascular status impact on gait performance (3,6), there is a paucity of studies examining the association of \textit{APOE} genotypes with gait. The few studies that have investigated the role of the \textit{ε4} allele on mobility used self-reported function or composite motoric measures and had mixed results (7–10). Presence of \textit{ε4} allele was associated with more rapid motoric decline in older adults (7) and worsening physical function in older male twins (9). On the other hand, the \textit{ε4} allele was not associated with risk of disability, mobility loss, or frailty in four population-based cohorts (10–13).

We undertook a prospective cohort study in 627 community-residing older adults participating in the Einstein Aging Study (EAS) to test the hypothesis that presence of \textit{APOE} \textit{ε4} allele status is associated with increased rate of decline in gait speed. A greater risk of motoric decline has been reported in older men (7,14). Higher risk of decline on several cognitive processes related to gait in male \textit{APOE} \textit{ε4} allele carriers has been reported (15). Furthermore, gender differences in \textit{APOE} allele effects on vascular disease pathways that increase risks of gait decline are known (16). Hence, we conducted analyses to account for the influence of gender on gait decline. Gait speed is used to describe functional recovery in older adults and predicts disability and survival (17,18). Therefore, we also examined the association of \textit{APOE} allele status with risk of developing disability.

\textbf{METHODS}

\textbf{Study Population} We conducted a prospective cohort study based on the EAS. The primary aim of the EAS is to identify risk factors for dementia. Study design has been reported (19). In
brief, potential participants (aged 70 years and older) identified from Bronx County population lists were contacted by letter explaining the study and then by telephone. The telephonic interview included verbal consent, medical history, and cognitive screeners. Exclusion criteria included severe auditory-visual loss, bed bound, and institutionalization. Following the interview, an age-stratified sample of participants who matched on a computerized randomization procedure was invited for further evaluation at our research center. Participants returned annually for clinical, neuropsychological, and mobility assessments. In between visits, participants were contacted by telephone every 2–3 months to assess function. Informed consent was obtained according to protocols approved by the local institutional review board.

This substudy began on February 2002 when we started systematically ascertaining gait and mobility in our cohort (20) and ended in February 2012. Blood collection and genotyping were started in the EAS in 2004–2005. Of the 1,164 EAS participants evaluated during the 120-month study period, 923 had gait assessments and 627 APOE genotyping. The 627 participants with gait and genotype assessments formed the study sample. Remaining EAS participants were not genotyped due to absent blood collection, or samples were not processed for genetic studies. Individuals with and without APOE genotyping were not significantly different in age, sex, race, and education. Compared with the 627 individuals with gait and genotyping, the 83 participants with genotyping but no gait assessments were slightly less educated (13.9 vs 12.8 years, \( p = .006 \)) but were similar in terms of age, sex, and race.

Gait

Gait speed was measured at baseline and annual follow-up visits using a computerized walkway with embedded pressure sensors (GAITRite, CIR systems, PA). GAITRite is widely used and has excellent validity and reliability (21). Participants are asked to walk on the walkway at their “usual pace” in a quiet well-lit room wearing comfortable footwear and without any attached monitors. Until July 2008, participants walked for two trials on a walkway with 15 feet of recording surface. After July 2008, assessments were done for one trial on a walkway with 20-foot recording surface. Correlation for gait speed measured on the two walkways in 20 EAS participants was excellent (Pearson \( r = .94 \)). We have ascertained excellent intraclass correlation coefficient (0.96) for gait speed between trials completed 2–3 hours apart on the same walkway (22). Start and stop points were marked by white lines on the floor and included three feet (four feet for newer walkway) from the edge of the recording surface to account for initial acceleration and terminal deceleration.

Disability

We instituted systematic assessment of disability in 2004 (22). During clinic visits and interim 2–3 monthly telephone interviews, participants were assessed for disability with a validated scale (23). Disability was defined as needing assistance with or inability to perform any one of seven activities of daily living: bathing, walking inside home, chair rise, dressing, feeding, toileting, and grooming. To exclude transient episodes, duration of disability had to be at least 6 months or result in change in living situation such as nursing home placement (23).

Genotyping

A phlebotomist did blood draws in all participants who provided consent (19). DNA was either extracted from whole blood or isolated from buffy coat stored at −70°C using the Puregene DNA Purification System (Gentra System, Minneapolis). Amplification and sequencing primers for genotyping of target APOE loci (dbSNP ID: rs7412 and rs429358) were designed using PSQ version 1.0.6 software (Biotage); in each case, the reverse primer was biotinylated. Genotyping was performed using a Pyrosequencing PSQ HS 96A system (http://www.pyrosequencing.com) according to manufacturer’s instructions.

Other Covariates

Presence or absence of self-reported vascular diseases (diabetes, heart failure, hypertension, angina, myocardial infarction, or strokes) and other medical illnesses (depression, Parkinson’s disease, chronic obstructive lung disease, or severe arthritis) was used to calculate a summary illness index (range 0–10) (20). Participants reported level of physical activity (21). An extensive cognitive battery was administered. For this investigation, we report general mental status measured by the Blessed Information Memory Concentration test (24). All information (except gait) was reviewed at consensus case conferences to assign dementia diagnoses.

Data Analysis

Baseline characteristics were compared using descriptive statistics. To determine the longitudinal association of APOE \( \varepsilon 4 \) allele with gait speed decline, linear mixed effects models controlled for age, sex, race, education, and medical illness were applied to 627 participants (model 1). The cohort was categorized into those with APOE \( \varepsilon 4 \) alleles (4/4, 2/4, and 3/4) and those without (3/3 and 3/2) who served as the reference group. Associations are reported as parameter estimates with 95% CI. A random intercept was included in the model to allow entry point to vary across individuals. “Time” represents rate of change in gait speed over time among APOE \( \varepsilon 4 \) allele noncarriers with baseline age 80, 12 years education, and values 0 for all covariates in the model. An interaction between APOE \( \varepsilon 4 \) allele and “time” was included to model the effect of the genotype on gait speed decline, which is the difference in slopes between \( \varepsilon 4 \).
carriers and noncarriers. In model 2, we added the following covariates to model 1: cognitive status (Blessed test) (24), low physical activity, and smoking. Another model (model 3) was fit with cholesterol added to the covariates in model 2. As serum cholesterol data were only available in 380 participants, we applied a multiple imputation approach. The missing cholesterol was imputed based on its observed association with age, sex, race, education, illness index, smoking status, physical activity level, APOE, and baseline gait speed. The imputation was repeated five times to obtain five imputed data sets. For each imputed data, gait decline, and incident disability (see below) analyses were performed, and estimates of the effect of APOE, further adjusted for cholesterol, were obtained. The final parameter estimate was the average of the five estimates, with the variance estimated by taking into account both within and between imputation variability.

We conducted sex-stratified analyses using above models. African-American EAS participants had slower gait at cross-section compared with whites (25). However, due to the low number of African Americans with longitudinal gait data, race effects were only examined in white individuals.

We selected change in stride length variability as an additional gait measure to examine genotype effects. We have established reliability (26), effect sizes (26), and predictive validity of stride length variability (standard deviation) for falls and dementia (20,21). Although GAITRite generates several other variables (20,21), the clinical significance of change in these other variables has not been established, limiting interpretation of any findings.

We examined effect of the ε4 allele on risk of developing disability using Cox proportional-hazards models to compute hazard ratios (HR) with 95% CI adjusted for age, sex, race, education, and medical illness.

Given the associations of the APOE ε4 allele with dementia (4,27), we repeated analyses for gait speed decline and disability in model 2 excluding 44 cases with prevalent dementia and the 37 individuals who developed incident dementia within 2 years of baseline. Regression diagnostics for all models were examined analytically and graphically and were adequately met. All statistical analyses were performed using SAS version 9.2 (SAS Institute, Cary, NC) and S-Plus 8.0 (Insightful). SAS 9.2 procedures MI and MIANALYZE were used to obtain multiple imputation estimates.

RESULTS

Study Population

The 627 eligible participants included 235 men (37.5%) and 392 women (62.5%). Mean age was 79.4±5.2 years. Median follow-up time was 3.0 years (maximum 9.3) with 484 participants (77.2%) completing one or more follow-up visits. There were 41 deaths during the study period, 15 occurring before the first follow-up visit. Proportions of various APOE alleles were 3/3, 61.2%; 3/4, 19.0%; 4/4, 1.6%; 2/4, 3.5%; 2/3, 13.6%; and 2/2, 1.1%. The proportion of any APOE ε4 allele (24.1%) was similar to other racially and ethnically diverse U.S. urban cohorts (7). Genotype frequencies did not differ from Hardy–Weinberg equilibrium (χ² = 4.167, df = 3, p = .24).

APOE ε4 allele carriers and noncarriers were similar in baseline characteristics except for race and physical activity (Table 1). African-American participants had higher prevalence of APOE ε4 carriers (34.7% vs 20.5%, p < .001), included more women (74.7% vs 56.7%, p < .001), were younger (mean 78.7 vs 79.8 years, p = .02) and had borderline higher illness index scores (1.3 vs 1.2, p = .06) compared with white participants.

APOE and Gait Decline

The mean rate of gait speed decline in the cohort was 2.56 cm/s per year. At baseline, there was no difference in gait speed between participants with and without the ε4 allele (Table 1). Table 2 shows that the APOE ε4 allele was not associated with gait speed decline in the entire sample. The ε4 allele was also not associated with gait speed decline in model 2 (estimate: −0.23, 95% CI −1.01 to 0.55). The result was similar when cholesterol was further adjusted in model 3 (estimate: −0.21, 95% CI −0.98 to 0.56).

Influence of Sex and Race

Test of interaction between the APOE ε4 allele and sex on gait speed decline was significant (model 2: estimate: = −1.70, 95% CI −3.33 to −0.07, p = .04). Significant interactions of the ε4 allele with age, education, race, and illness were not seen (data not shown).

Spaghetti plots of gait speed decline by sex and APOE status are shown in Figure 1. In the sex-stratified analysis (Table 2), men with ε4 allele showed more rapid gait speed decline than male noncarriers (estimate: −1.16, 95% CI −2.31 to −0.01). In women, differences were not significant (estimate: 0.44, 95% CI: −0.61 to 1.48). Results were similar in model 2.

The ε4 allele was associated with increased gait speed decline in the analysis restricted to whites (estimate: −1.48 cm, 95% CI 0.38–2.92). The magnitude of the association was larger in white males (estimate: −1.78, 95% CI 0.49–3.08).

Disability

Baseline for this analysis was 2004 when we instituted disability assessments in the EAS. We included 554 participants without prevalent disability and with follow-up. There were 92 incident disability cases over a median follow-up of 36.5 months. Table 3 shows that the ε4 allele was not associated with risk of disability (HR: 0.76, 95% CI 0.41–1.43) overall. The ε4 allele was associated with
increased risk of disability in the 212 men (HR: 3.72, 95% CI 1.44–9.59) but not in the 342 women (HR: 0.77, 95% CI 0.42–1.48). Results were similar in model 2. There was no significant interaction of the ε4 allele with race. Survival plots in Figure 2 shows disability free survival rate in participants with and without ε4 allele. Twenty-two (15%) participants with ε4 allele and 70 (17%) noncarriers developed disability during median follow-up of 2.8 and 3.1 years, respectively.

**Sensitivity Analysis**

In analysis excluding prevalent and incident dementia cases in model 2 (adjusted for Blessed test) (24), the ε4 allele did not predict gait decline overall (estimate: 0.55, 95% CI −0.47 to 1.57, p = .29) but predicted gait decline in men (estimate: −1.63, 95% CI −3.19 to −0.07, p = .03). Similarly, the ε4 allele did not predict disability overall (HR: 0.71, 95% CI 0.37–1.41) but predicted disability in men (HR: 3.09, 95% CI 1.71–8.13).

The mean rate of change in stride length variability was −0.04 SD/year (men: −0.08 SD/year). APOE ε4 allele was not associated with decline in stride length variability overall (estimate: −0.07, 95% CI −0.92 to 0.79) or in men (estimate: −0.01, 95% CI −0.72 to 0.74).

Our preliminary analysis did not show a significant effect of the APOE ε2 allele on gait speed decline. Hence, these results are not reported.
**DISCUSSION**

Our findings show that presence of the \textit{APOE} ε4 allele was not associated with increased risk of gait speed decline in the overall EAS cohort. However, ε4 allele was associated with more rapid gait speed decline in older men but not in women. Older men with the ε4 allele had a 1.16 cm/s increased rate of gait speed decline per year compared with noncarriers. The associations remained robust even after adjustments for multiple potential confounders such as vascular disease, neurological diseases including dementia, cognition, and physical activity levels. We also examined risk of developing disability, a clinical outcome predicted by gait speed in our cohort (28). Our findings show that older male APOE ε4 genotype carriers not only had greater gait speed decline (by 46%) than noncarriers but also were at increased risk of developing disability (HR: 3.7). To our knowledge, this is the first study to report the effect of \textit{APOE} genotype on gait decline in aging. Our results are supported by studies that have reported an association of the ε4 allele with decline on composite motoric indices with gait speed as one of the components (7,9).

---

**Table 3. Effect of \textit{APOE} ε4 on Risk of Incident Disability Adjusted for Age, Sex, Race, Education and Medical Illnesses in the Overall Cohort as well as Stratified by White Ethnicity and Sex**

<table>
<thead>
<tr>
<th></th>
<th>Overall (N = 554)</th>
<th>Overall (N = 373)</th>
<th>White (N = 212)</th>
<th>White (N = 166)</th>
<th>White (N = 342)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR (95% CI)</td>
<td>0.76 (0.41, 1.43)</td>
<td>0.76 (1.04, 1.68)</td>
<td>1.43 (0.67, 3.05)</td>
<td>1.43 (0.67, 3.05)</td>
<td>0.77 (0.42, 1.48)</td>
</tr>
<tr>
<td>p value</td>
<td>p = .40</td>
<td>p = .007</td>
<td>p = .35</td>
<td>p = .35</td>
<td>p = .46</td>
</tr>
</tbody>
</table>

Notes: 
HR = Hazard ratio.
Disability was defined as needing assistance with or inability to perform any one of seven activities of daily living for at least 6 months or institutionalization; activities include bathing, walking inside home, chair rise, dressing, feeding, toileting, and grooming.

---

Figure 1. Gait speed decline by apolipoprotein E (APOE) status and gender. The black lines represent gait speed measured in individual participants at baseline and follow-up visits. The red line shows the fitted average gait speed as a function of time.
The association of the \( APOE \varepsilon4 \) allele with risk of disability mirrored that for gait speed with no main effect but a significant risk in men. Older men with the \( APOE \varepsilon4 \) allele had an over threefold risk of developing disability compared with male noncarriers. Previous cross-sectional studies have had mixed results with both significant (29) and non-significant (30) associations of the \( \varepsilon4 \) allele reported with activities of daily living. Although four prospective population-based cohorts reported a lack of association of the \( \varepsilon4 \) allele with functional decline (10–13), not all examined sex effects. The \( \varepsilon4 \) allele did not predict incident self-reported mobility disability (10). Moreover, there were no significant interactions between sex and \( APOE \) status on mobility outcomes (10). Another study in an African-American cohort did not find a main effect; however, women with the \( \varepsilon4 \) allele were at higher risk of functional decline (11). No significant association of \( \varepsilon4 \) allele with study outcomes was seen in African Americans in our cohort (data not shown). The low number of African Americans (\( n = 170 \)) and smaller proportion of men (\( n = 43 \)) in this subgroup limit statistical power. Differences in sample sizes, variable disability definitions, lack of systematic disability follow-up, and racial composition may also explain divergent results.

As this epidemiological study was designed to generate hypotheses regarding genotype–phenotype associations, any discussion of biological mechanisms to explain this effect is admittedly speculative. The \( \varepsilon4 \) allele predicted decline in gait speed but not stride length variability in men suggesting specificity of genotype effects on discrete gait pathways. The \( \varepsilon4 \) allele may increase risk of gait speed decline and disability through several mechanisms including its influence on peripheral nerves (8), cognition (4), or vascular disease (5). \( APOE \varepsilon4 \) allele carriers have been reported to show deficits on cognitive processes associated with gait such as divided attention (31) and spatial navigation (32) in some but not all studies (33). Our results were not different after adjusting for cognitive status and excluding individuals with prevalent dementia and those who developed dementia within the first 2 years. The higher prevalence of vascular disease in men, as well as gender differences in \( APOE \) allele effects on lipid metabolism, has been suggested to account for higher dementia risk in older male carriers (34). Adjustments for vascular risk factors including serum cholesterol did not materially change our results. However, other quantitative or biological measures of vascular disease may provide additional information.

As with any observational study, cautious interpretation is advisable. Our cohort while broadly reflective of the community-residing Bronx elderly population is not representative, and our findings need to be verified in other populations. Our sample size was comparable with other longitudinal mobility studies, but we might have seen a significant association in women with larger samples. Nonetheless, comparisons of the magnitude of estimates between sexes suggest that the genotype effects on our study outcomes are stronger in men. Not all EAS participants had gait assessments and genotyping as these measures were introduced after the study inception. Disability assessments were not done past the point participants met criteria. Hence, we were unable to comment on the effect of genotype on change in disability patterns.

Our results suggest that \( APOE \) isoforms influence risk of gait decline and risk of disability in older men. These results should be considered preliminary, and further studies are required to validate our results, elucidate biological pathways, and determine whether interventions targeted at \( APOE \varepsilon4 \) allele carriers may reduce gait decline and disability.
References