APOE 4 and Hip Fracture Risk in a Community-Based Study of Older Adults

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OBJECTIVES: To investigate whether the APOE 4 allele was associated with increased risk of hip fracture in an older community-based sample and whether such an increased risk was independent of dementia and history of falling.

DESIGN: Case-control study nested within a prospective community study.

SETTING: The Monongahela Valley Independent Elders Survey (MoVIES), an ongoing prospective community study of older adults in southwestern Pennsylvania.

PARTICIPANTS: A total of 899 MoVIES participants (63.9% women; mean age, 76.2 years, SD = 4.9 years), who provided both information on hip fractures and blood samples for genotyping.

MEASUREMENTS: Interview questions regarding hip fractures and falls, polymerase chain reaction to determine APOE genotype, and clinical assessment using a standardized protocol to determine the presence or absence of dementia.

RESULTS: Twenty-five subjects reported having hip fractures in the year preceding screening interviews. Subjects with one or two APOE 4 alleles were twice as likely as subjects without an APOE 4 allele to report hip fractures (age-adjusted OR = 2.1, 95% CI: 0.9–4.7). Based on multivariate analysis, subjects with a history of falling were more likely to report hip fractures (OR = 4.7, 95% CI: 2.1–10.8). After adjusting for history of falls and diagnosis of dementia, subjects with an APOE 4 allele were still twice as likely to report hip fractures (adjusted OR = 2.1, 95% CI: 0.9 - 4.7).

CONCLUSIONS: The APOE 4 allele appears to be a risk factor for hip fracture, independent of the effect of dementia and falling. Theoretically, this may be mediated by alterations in vitamin K metabolism. Caution should be used in interpreting these results, because the 95% confidence intervals for the odds ratios include 1. J Am Geriatr Soc 47:1342–1345, 1999.

Key words: hip fracture; apolipoprotein E, *APOE*; community study

Apolipoprotein E (apo E) is involved in cholesterol metabolism[1] and in repair and remodeling of nerve cells.[2] There are three apo E isoforms (apo E2, apo E3, and apo E4) that are encoded by three *APOE* alleles (designated *APOE 2*, *APOE 3*, and *APOE 4*, respectively) on chromosome 19. The *APOE 4* allele is a well-established risk factor for atherosclerosis,[3] coronary heart disease,[4] and Alzheimer's disease.[5–8]

The *APOE 4* allele has recently been found to be associated with an increased risk of bone fracture in hemodialysis patients[9] and with an increased risk for hip and wrist fractures and accelerated bone loss in older women.[10] In postmenopausal Japanese women, the apo E4 phenotype has also been associated with lower bone mineral density.[11]

The purpose of this study was to investigate whether the *APOE 4* allele was associated with increased risk of hip fracture in an older community-based sample and whether such an increased risk was independent of dementia and history of falling.

METHODS

Study Design

This case-control study was nested within the ongoing Monongahela Valley Independent Elders Survey (MoVIES). MoVIES is a prospective community study of 1681 older adults in the mid-Monongahela Valley region of southwestern Pennsylvania. The analyses reported here are based on 899 members of the MoVIES cohort who provided a blood sample for *APOE* genotyping between 1994 and 1997 and provided information about occurrence of hip fractures.

Sampling and recruitment for MoVIES has been described in detail previously.[12,13] Briefly, the MoVIES cohort consisted of a random sample of 1422 persons, drawn primarily from voter registration lists, and 259 volunteers who lived in the same area. To be eligible to participate, subjects had to be aged 65 years or older, noninstitutionalized at time of study entry (between 1987 and 1989), fluent in English, and have at least a sixth-grade education.

The study was approved by the Health Sciences Institutional Review Board at the University of Pittsburgh. All subjects or their next-of-kin provided informed consent to participate in the study.

Data Collection

APOE Genotyping

Collection of blood samples for *APOE* genotyping from MoVIES subjects began in January 1994 and was completed in October 1997. DNA was isolated from peripheral blood

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leukocytes (buffy coat) using the QIAmp kit (QIAGEN Inc., Chatsworth, CA). Genotyping was performed using a polymerase chain reaction protocol as described previously.[14]

History of Hip Fracture and Falls

At study entry and during each biennial follow-up wave, all MoVIES participants were asked to complete a risk factor screening interview. Questions regarding history of fractures and falls were asked of all MoVIES participants as part of the biennial risk factor screening, starting at the beginning of the third wave in 1991. During waves three, four, and five, subjects were asked whether they had experienced a hip fracture in the preceding year. They were also asked if they had fallen in the past year, and, if so, how many times. When necessary, and if available, proxy informants were asked to provide answers to these and other risk factor screening questions.

Diagnosis of Dementia

Identification of dementia cases was based on a multistage process that has been described previously.[15] At entry into the MoVIES study and during each biennial follow-up screening wave, all participants were screened with a battery of standard neuropsychological tests. Clinical (diagnostic) assessment was offered to all subjects who met the operational criteria for cognitive impairment and/or cognitive decline. Presence or absence of dementia was determined according to the *Diagnostic and Statistical Manual*, Volume III, Revised (DSM-III-R);[16] stage of dementia, according to the Clinical Dementia Rating (CDR); and probable etiology of dementia (probable Alzheimer's disease (AD), Possible AD, or other) according to the National Institute of Neurological and Cognitive Disorders and Stroke–Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) work group criteria[17] and the Consortium to Establish a Registry for Alzheimer's Disease (CERAD) protocol for non-AD dementia.[18] Final diagnoses of dementia were made by consensus among investigators and staff using all diagnostically relevant information, including clinical, historical, and laboratory data and medical records.

Data Analysis

During one to three screening interviews, each subject provided information about hip fracture in the preceding year, producing a total of 2540 observations. To analyze correlated data (repeated screenings for fractures in the same person), Generalized Estimating Equations (GEE) regression[19,20] was used. We first used GEE to calculate age-adjusted odds ratios (ORs) for hip fracture for subjects with one or two *APOE 4* alleles, compared with subjects without an *APOE 4* allele. Then indicator variables were added into the GEE model to determine whether the association between hip fracture and the presence of an *APOE 4* allele was independent of history of falling and of dementia. A subject was considered to have a history of falling if the subject reported two or more falls in the year before hip fracture data was obtained. A subject was considered to be demented if the subject was diagnosed with dementia (CDR stage ≥ 0.5) with onset before the risk factor screening. Because the association of the *APOE 4* allele with AD may be stronger than the association with all dementias,[6] we repeated the analyses substituting possible or probable AD (CDR stage ≥ 0.5) for all dementias.

In a previous study, fracture risk was highest in subjects with the *APOE* 3/4 or *APOE* 4/4 genotype, lowest in subjects with the *APOE* 2/2 or *APOE* 2/3 genotype, and intermediate for subjects with the *APOE* 3/3 genotype.[9] In our sample, 23 hip fractures occurred in subjects with an *APOE* 3/3 or *APOE* 3/4 genotype, whereas only two occurred in subjects with the *APOE* 2/3 genotype. Therefore, we were not able to analyze the risk of hip fracture associated with the *APOE 2* allele. However, to isolate the effect of the *APOE 4* allele, we repeated the analyses restricting the sample to subjects with the *APOE* 3/3 or *APOE* 3/4 genotypes.

Because the frequency of the *APOE 4* allele has been found to be higher[21] and the risk of hip fracture lower[22] in blacks than in whites, we repeated the analyses restricting the sample to 883 white subjects with *APOE* genotypes and hip fracture data.

RESULTS

The study population numbered 899 (25 subjects who reported a hip fracture and 874 controls who did not), was 63.8% women, 98.2% white, and had a mean age of 76.2 years (SD = 4.9 years) when first asked about hip fracture occurrence. Hip fracture cases were more likely to be women (80.0% vs 63.4%, $P = .09$) and to be white (100% vs 98.2%, $P = .50$), but neither of these differences was statistically significant. Allele frequencies in the entire study population were as follows: *APOE 2*, 0.068; *APOE 3*, 0.819; and *APOE 4*, 0.112. Among the study population, 21.2% had one or two *APOE 4* alleles. The genotype distribution was in Hardy-Weinberg equilibrium. Compared with the original MoVIES cohort members who were not included in this study, study participants were more likely to be women (63.8% vs 50.8%, $P = .001$), to be white (98.2% vs 95.8%, $P = .003$), and to have been younger at entry into the MoVIES study (71.9 ± 4.9 years vs 75.2 ± 6.5 years, $P = .001$).

Subjects with one or two *APOE 4* alleles were twice as likely as subjects without an *APOE 4* allele to report hip fractures (age-adjusted OR = 2.10, 95% CI: 0.94–4.70). Results were similar when the analysis was restricted to white subjects (OR = 2.09, 95% CI: 0.93–4.67) and when the analysis was restricted to subjects with the APOE 3/3 and APOE 3/4 genotypes (OR = 2.33, 95% CI: 1.02–5.33). These results only reached statistical significance when the analysis was restricted to subjects with the APOE 3/3 and APOE 3/4 genotypes.

Based on multivariate GEE regression analysis, which included all the variables listed in Table 1, subjects who reported two or more falls in the year before screening were significantly more likely to report hip fractures during the previous year, independent of *APOE* genotype or diagnosis of dementia. Based on the same model, subjects with at least one *APOE 4* allele were twice as likely to report hip fractures, even after adjusting for history of falls and diagnosis of dementia. However, this result did not reach statistical significance. Results were similar when the analyses were restricted to white subjects and when diagnosis of AD was substituted for diagnosis of dementia.

DISCUSSION

In the MoVIES cohort, subjects with one or two *APOE 4* alleles were approximately twice as likely to report a hip fracture as were subjects without an *APOE 4* allele. This association appears to be independent of history of falling

Table 1. Multivariate GEE Regression Model for Report of Hip Fracture

	All subjects		Subjects with *APOE* 3/3 or 3/4 genotype	
	OR	95% CI	OR	95% CI
1 or 2 *APOE 4* alleles	2.10	0.93–4.74	2.39	1.03–5.55
Diagnosis of dementia	1.69	0.67–4.22	1.41	0.53–3.70
History of 2 or more falls	4.70	2.05–10.77	4.79	1.98–11.61
Age (1-year increase)	1.10	1.03–1.17	1.10	1.03–1.18

and diagnosis of dementia or AD. Caution should be used in interpreting these results, because the 95% confidence intervals for the odds ratios include 1. Lack of statistical significance may result from the small number of hip fractures that occurred in our study population.

Our study confirms the findings of the Study of Osteoporotic Fractures (SOF), in which women with an *APOE 4* allele were twice as likely to suffer from hip fracture as were women without an *APOE 4* allele, independent of age, mental status, falling, bone mineral density, weight, and functional status.[10] Our study extends the SOF findings in that the MoVIES study includes both sexes and detailed diagnostic data on both dementia and AD, whereas mental status in SOF was limited to cognitive screening data.

The *APOE 4* allele is a well-established risk factor for Alzheimer's disease.[5–8] AD patients have been found to be at increased risk for hip fracture, possibly because of increased likelihood of falling, gait abnormalities, and diminished reflexes.[23] However, MoVIES subjects with dementia or AD were only at a slightly (nonsignificant) increased risk for hip fracture. Adjusting for dementia and history of falling did not change the association between the presence of an *APOE 4* allele and hip fracture.

Theoretically, the association between the *APOE 4* allele and increased risk of hip fracture may be mediated by alterations in vitamin K metabolism. Vitamin K, which shares metabolic pathways with triglyceride-rich lipoproteins (chylomicrons and chylomicron remnants), may be cleared from the bloodstream faster in individuals with an *APOE 4* allele than in subjects without an *APOE 4* allele. Vitamin K1 levels in hemodialysis patients with the apo E3/4 and E4/4 phenotypes were found to be less than half those of patients with the E3/3 phenotype.[24] Patients with hip fractures or spinal crush fractures have been shown to have very low concentrations of circulating vitamin K1 and vitamin K2.[25] Vitamin K supplementation has been shown to increase serum osteocalcin and bone alkaline phosphatase (markers of bone formation) and may reduce urinary calcium and hydroxyproline excretion (markers of bone resorption).[25]

Our results may be affected by certain study limitations. Hip fracture status was based on self-report of hip fracture in the year before the risk factor screening interview. However, investigators in SOF found self-report of hip fracture to be accurate, with a relatively low false positive rate.[26] Subjects with an *APOE 4* allele are at increased risk for AD, which may make them or their informants less likely to reliably report a hip fracture that occurred. If so, this would bias study findings toward the null hypothesis, making it more difficult to detect an association between the presence of an *APOE 4* allele and hip fracture. Our results are based on a large total number of observations (2540) but a small number of hip fractures (25); thus, they should be viewed as preliminary until they are replicated in studies with more confirmed hip fracture events.

This study examined the relationship between presence of an *APOE 4* allele and hip fracture risk among participants in a community-based study of dementia and cognitive impairment. In this population, the *APOE 4* allele seems to be a risk factor for hip fracture, independent of history of falling and diagnosis of dementia. Potentially this increased risk may be mediated by alterations in vitamin K metabolism. Such a possibility may have clinical implications for patients taking anticoagulant therapy.

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REFERENCES

1. Mahley RW. Apolipoprotein E: Cholesterol transport protein with expanding role in cell biology. Science 1988;240:622–630.
2. Poirier J. Apolipoprotein E in the brain and its role in Alzheimer's disease. J Psychiatry Neurosci 1996;21:128–134.
3. Wang XL, McCredie RM, Wilcken DEL. Polymorphisms of the apolipoprotein E gene and severity of coronary artery disease defined by angiography. Arterioscler Thromb Vasc Biol 1995;15:1030–1034.
4. Wilson P, Schaefer E, Larson M, Ordovas J. Apolipoprotein E alleles and risk of coronary disease: A meta-analysis. Arterioscler Thromb Vasc Biol 1996;16:1250–1255.
5. Evans DA, Beckett LA, Field TS et al. Apolipoprotein E ϵ4 and incidence of Alzheimer disease in a community population of older persons. JAMA 1997; 277:822–824.
6. Myers RH, Schaefer EJ, Wilson PWF et al. Apolipoprotein E ϵ4 association with dementia in a population-based study: The Framingham study. Neurology 1996;46:673–677.
7. Saunders AM, Strittmatter WJ, Schmechel D et al. Association of apolipoprotein E allele ϵ4 with late-onset familial and sporadic Alzheimer's disease. Neurology 1993;43:1467–1472.
8. Strittmatter WJ, Saunders AM, Schmechel D et al. Apolipoprotein E: High-avidity binding to beta-amyloid and increased frequency of type 4 allele in late-onset familial Alzheimer disease. Proc Natl Acad Sci U S A 1993;90: 1977–1981.
9. Kohlmeier M, Saupe J, Schaefer K, Asmus G. Bone fracture history and prospective bone fracture risk of hemodialysis patients are related to apolipoprotein E genotype. Calcif Tissue Int 1998;62:278–281.
10. Cauley JA, Zmuda JM, Yaffe K et al. Apolipoprotein E polymorphism: A new genetic marker of hip fracture risk—the Study of Osteoporotic Fractures. J Bone Miner Res 1999;14:1175–1181.
11. Shiraki M, Shiraki W, Aoki C et al. Association of bone mineral density with apolipoprotein E phenotype. J Bone Miner Res 1997;12:1438–1445.
12. Ganguli M, Belle S, Ratcliff G et al. Sensitivity and specificity for dementia of population-based criteria for cognitive impairment: The MoVIES project. J Gerontol 1993;48:M152–161.

13. Ganguli M, Lytle ME, Reynolds MD, Dodge HH. Random versus volunteer selection for a community-based study. J Gerontol 1998;53:M39–46.
14. Kamboh MI, Aston CE, Hamman RF. The relationship of APOE polymorphism and cholesterol levels in normoglycemic and diabetic subjects in a biethnic population from the San Luis Valley, Colorado. Atherosclerosis 1995; 112:145–159.
15. Ganguli M, Ratcliff G, DeKosky ST. Cognitive test scores in community-based older adults with and without dementia. Aging Mental Health 1997;1: 176–180.
16. American Psychiatric Association. Diagnostic and Statistical Manual of Mental Disorders. Washington, DC: American Psychiatric Association, 1987.
17. Morris JC. The Clinical Dementia Rating (CDR): Current version and scoring rules. Neurology 1993;43:2412–2414.
18. Fillenbaum GG, Heyman A, Huber MS et al. The prevalence and 3-year incidence of dementia in older black and white community residents. J Clin Epidemiol 1998;51:587–595.
19. Zeger SL, Liang KY. Longitudinal data analysis for discrete and continuous outcomes. Biometrics 1986;42:121–130.
20. Liang KY, Zeger SL. Longitudinal data analysis using general linear models. Biometrika 1986;73:13–22.
21. Kamboh MI, Sepehrnia B, Ferrell RE. Genetic studies of human apolipoproteins: VI. Common polymorphism of apolipoprotein E in blacks. Dis Markers 1989;7:49–55.
22. Kannus P, Parkkari J, Sievanen H et al. Epidemiology of hip fracture. Bone 1996;18(suppl): S57–63.
23. Melton LJ, Beard CM, Atkinson EJ, O'Fallon WM. Fracture risk in patients with Alzheimer's disease. J Am Geriatr Soc 1994;42:614–619.
24. Kohlmeier M, Salomon A, Saupe J, Shearer J. Transport of vitamin K to bone in humans. J Nutr 1996;126(suppl): S1192–1196.
25. Vermeer C, Gijsbers MG, Craciun M et al. Effects of vitamin K on bone mass and bone metabolism. J Nutr 1996;126(suppl): S1187–1191.
26. Nevitt MC, Cummings SR, Browner WS et al. The accuracy of self-report of fractures in elderly women: Evidence from a prospective study. Am J Epidemiol 1992;135:490–499.

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