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Association of Apolipoprotein E Genotype and Alzheimer Disease in African Americans

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Abstract

Background—Alzheimer disease (AD) is the most frequent cause of dementia. Even though the incidence of AD in the African American population is similar to or higher than that in persons of European descent, AD in African Americans is understudied. Identification of genetic risk factors in African Americans is essential for understanding the etiology of AD.

Objective—To determine the effect of apolipoprotein E (*APOE*) genotype on the risk of AD in elderly African Americans.

Design—Population-based longitudinal study of AD.

Setting—Indianapolis, Ind.

Participants—African Americans 65 years and older.

Main Outcome Measures—*APOE* genotype and diagnosis of AD.

Results—The *APOE* genotype was determined in 1822 samples. Of these, 690 were clinically evaluated: 318 were normal, and 162 had a diagnosis of AD. The presence of *APOE* ε4 was significantly associated with increased risk of AD (ε3/ε4: OR, 2.32; 95% confidence interval [CI], 1.41–3.82; and ε4/ε4: OR, 7.19; 95% CI, 3.00–17.29, compared with the ε3/ε3 genotype). There was also a significant protective effect with *APOE* ε2 (ε2/ε2 and ε2/ε3: OR, 0.42; 95% CI, 0.20–0.89).

Conclusions—These findings are in marked contrast to the lack of association between *APOE* and AD in the Ibadan, Nigeria, sample of this project. These results suggest that other genetic factors and different environmental influences may play a role in the risk for AD in individuals of African ancestry.

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Alzheimer Disease (AD) accounts for a large part of public health spending in developed societies[1] and is becoming an economic burden in developing countries.[2] The incidence of AD in African Americans is similar to or higher than that in persons of European descent.[3–9] A meta-analysis that included data from several ethnic groups showed that the ε4 allele of the apolipoprotein E gene (*APOE*) is a major susceptibility factor in the development of AD.[10] In many of these populations, a dose-dependent relationship has been shown between *APOE* ε4 and the risk of AD, but this relationship has less accord among African American populations.[3,11–14]

Our group has previously published findings on the relationship between *APOE* genotype and the risk for AD in the Indianapolis-Ibadan Dementia Study, a longitudinal, cross-cultural study of AD in African Americans living in Indianapolis, Ind, and Yoruba living in Ibadan, Nigeria.[12,13,15] The initial findings showed a significant association between *APOE* ε4 and AD in African Americans[12] that was genotype dependent.[13] In the Yoruba, neither 1 nor 2 *APOE* ε4 alleles were associated with an increased risk for AD.[15]

Currently, 1822 African American subjects have been genotyped for *APOE*, and clinical assessments have been completed on 690 subjects, including 162 with AD. Herein we report our updated findings.

METHODS

STUDY DESIGN AND SUBJECTS

Data were derived from a population-based study on the prevalence and incidence of AD in 4102 African Americans 65 years and older living in Indianapolis. Only individuals who provided signed informed consent were studied. Race was determined by self-report using the question on race from the US Census. The original cohort of 2212 was enriched by enrolling new subjects who were 70 years or older in 2001. A detailed description of the study methods has been published.[7] The Indiana University (Indianapolis) Institutional Review Board has approved the study protocol. Data were collected from a baseline wave conducted in 1992–1993 and follow-up evaluations conducted 2, 5, and 8 years after baseline for the original cohort, and from a baseline wave for the newly enrolled subjects in 2001. At each wave a 2-phase design was used. In the first phase, study subjects were interviewed in their homes using the Community Screening Interview for Dementia.[16] Participants selected on the basis of their screening performance received a full diagnostic evaluation in the second phase.

At each evaluation wave, study participants were divided into 3 performance groups—good, intermediate, and poor—on the basis of their current screening scores and changes in their score from all previous waves. Cutoff points on change scores were derived so that approximately 5% of subjects with the worst change scores (most declines) were in the poor-performance group and approximately 8% of subjects with the next worst change scores were in the intermediate-performance group. The cross-sectional and longitudinal groupings were combined into one in which subjects were categorized by the worst of the 2 groupings. All subjects in the poor-performance group were chosen to be clinically assessed, ensuring that participants with the highest probability of having dementia would be diagnosed. Participants were randomly sampled from the intermediate-performance group until 50% had undergone clinical assessments, and from the good-performance group (weighted for 75% of those ≥75 years) until 5% had undergone clinical assessments.

Clinical assessments consisted of an informant interview, neuropsychological testing, and a physician examination. Clinicians were blinded to the performance group assignment and *APOE* genotype for all subjects. Diagnoses of normal, cognitive impairment, or dementia/

AD were made by a consensus of study physicians and neuropsychologists from Indianapolis and Ibadan. For a diagnosis of dementia, both *International Statistical Classification of Diseases, 10th Revision*,[17] and *Diagnostic and Statistical Manual of Mental Disorders, Revised Third Edition*,[18] criteria had to be met. For a diagnosis of probable or possible AD, we used the criteria of the National Institute of Neurological and Communicative Disorders and Stroke–Alzheimer's Disease and Related Disorders Association.[19]

APOE ANALYSES

DNA samples were extracted from blood spots collected on filter paper[20] or from fresh blood by standard protocols. *APOE* was genotyped by *Hha*I digestion of amplified products.[21]

STATISTICAL ANALYSES

Only subjects who had *APOE* genotyped and had undergone a clinical evaluation were included in this analysis. The AD group consisted of subjects who were diagnosed as having AD during the course of the study. Both prevalent and incident cases of AD were included because (1) the same diagnostic criteria and procedure were used throughout the study, (2) genetic constitution precedes the disease, and (3) this combination of cases would increase the power of our study. Individuals who were diagnosed as normal at the most recent clinical assessment formed the normal group. Subjects whose most recent diagnosis was cognitive impairment were excluded from this analysis. Demographic characteristics and *APOE* allele frequencies were compared between the subjects with and without a clinical diagnosis, with unpaired 2-tailed *t* tests for continuous variables and χ^2 tests for categorical variables. Similarly, the subjects with AD were compared with the normal subjects on these same characteristics. The subjects' age used in the analyses was the age at diagnosis for the subjects with AD and the age at the most recent clinical evaluation for the normal subjects. Because we used a nested case-control design and focused our investigation on the association between *APOE* genotype and AD, regular logistic regression models (after adjusting for age, sex, and years of formal education) were used to calculate the odds ratios (ORs) and 95% confidence intervals (CIs) for AD and for the *APOE* genotypes, using ε3/ε3 as the reference. It has been shown that regular logistic regression estimators on the exposure covariates apply under stratified sampling for case-control studies and that the bias is limited to the intercept term.[22,23] Logistic regression models including an additional interaction term between age and genotypes were also used to detect differential *APOE* effects at different ages. $P<.05$ was considered statistically significant.

RESULTS

A total of 1822 individuals were genotyped for *APOE*, of whom 690 had undergone a clinical evaluation. Of these, 318 were diagnosed as normal, 201 were diagnosed as demented, and of these 162 were diagnosed as having AD (69 subjects diagnosed at baseline and 93 during follow-up). The 171 subjects diagnosed as having cognitive impairment were excluded from the analyses.

When we compared *APOE*-genotyped subjects with (n=690) and those without (n=1132) a clinical evaluation, subjects with a clinical evaluation were significantly older (mean±SD age, 76.6±7.0 vs 75.8±5.7 years; $P=.008$), and less educated (mean±SD years of education, 9.4±3.2 vs 11.4±2.5 years; $P<.001$). There was no significant difference in sex. Also, the clinically evaluated subjects had a significantly greater amount of *APOE* ε4 alleles (23.4% vs 19.4%; $P=.004$) and a significantly smaller amount of ε2 alleles (9.5% vs 11.8%; $P=.03$).

There was no difference in the amount of ε3 alleles (67.1% vs 68.8%; *P*=.29). These differences were expected because we over sampled the poor-performance group.

Table 1 presents the baseline characteristics of the subjects with AD and the normal individuals. We found no significant difference in sex between those with AD and normal subjects (*P*=.64). Subjects with AD were significantly older than normal subjects (*P*<.001). In addition, subjects with AD had less education compared with normal subjects (*P*<.001). All subjects were followed up for similar lengths of time (*P*=.46).

Allele and genotype frequencies are given in Table 1. The *APOE* ε4 allele was significantly overrepresented in the AD group (*P*<.001) compared with normal subjects. Likewise, the ε2 and ε3 alleles were underrepresented in the AD group (ε2, *P*=.009; ε3, *P*<.001).

The logistic regression results of the association of *APOE* with AD, after adjusting for sex, age, and education, are given in Table 2. Having an ε2 allele in the presence of an ε3 allele significantly decreased the risk of AD (OR, 0.46; 95% CI, 0.21–0.99; *P*=.047) (data not shown). There were too few ε2 homozygotes to see a significant difference; however, combining those with ε2/ε2 and those with ε2/ε3 showed a protective effect (*P*=.02). In addition, having a single ε4 allele (in the absence of ε2) significantly increased one's risk for AD (*P*<.001). However, this risk increased 3-fold if the subject was homozygous for ε4 (*P*<.001). There were no significant interactions between age and genotype on the risk of AD (*P*=.60), indicating that the association between *APOE* genotype and AD does not vary by age. Separate logistic regression models in subsamples stratified by age group also gave similar results across age groups (data not shown).

COMMENT

In our current analysis including 162 subjects with AD, we found a significant relationship between *APOE* ε4 and AD in elderly African Americans. These updated results using the data from all the prevalence and incidence waves of our study are consistent with an earlier observation that *APOE* ε4 is a significant risk factor for AD.[12,13] In the pilot study,[12] having 1 or 2 ε4 alleles was significant for increased risk of AD; however, the number of samples was small. In the second report,[13] a dose-dependent association was observed, with only the ε4/ε4 genotype reaching significance. In the latter study, participants with cognitive impairment were incorporated into the normal group. However, even when those with cognitive impairment were removed from the normal group, the ε3/ε4 genotype still did not reach significance. When the participants with cognitive impairment were included in the normal group in the present study, the ε3/ε4 effect was of a lower magnitude but still significant (OR, 1.92; 95% CI, 1.23–3.01; *P*=.004) (data not shown). Our current analyses include more cases of AD than the 2 previous reports (31 subjects with AD and 54 normal subjects in the study by Hendrie et al[12] and 60 subjects with AD and 216 control subjects in the study by Sahota et al[13]).

Also in the present analysis, *APOE* ε2 was protective against AD in African Americans. This effect is similar to that observed in white and Japanese populations[10] and in a group of African American subjects from the southeastern United States.[11] This protective effect is some-times difficult to identify owing to the lower frequency of the ε2 allele in most populations.

Although we found an association between AD and *APOE*, this association did not change with increased age. As seen in white and Japanese subjects, the ε4 effect seems to be highest in individuals between the ages of 40 and 60 years and diminishes after age 70 years.[10] The Indianapolis-Ibadan study enrolled subjects 65 years and older in the original cohort and

subjects 70 years and older in the enrichment cohort. Therefore, younger subjects in whom *APOE* ε4 may have had a greater effect were missed.

Population-based studies among white and Japanese populations have found a significantly increased risk for AD with the *APOE* ε4/ε4 genotype (ORs of 14.9 for whites and 33.1 for Japanese) and a lesser but still increased risk with the *APOE* ε3/ε4 genotype (ORs of 3.2 for whites and 5.6 for Japanese).[10] However, this association is clearly not universal. Several other reports[3,11,14] suggest a less consistent relationship with *APOE* in African Americans. Longitudinal population-based studies of elderly African Americans living in New York, NY,[14] and Chicago, Ill,[3] showed no significant increase in the risk of AD with *APOE* ε4, similar to findings in the Yoruba[15] and Jamaican populations. [24] However, results from a clinic-based, case-control and family study examining African Americans from the southeastern United States showed a significant relationship between ε4 and AD risk with a magnitude similar to that of the present analysis.[11] A meta-analysis[10] suggested that there may be considerable heterogeneity in the pattern of association across individual data sets of African American subjects. Some of this difference may be due to small data sets in individual studies. However, our enriched Indianapolis cohort, along with the New York[14] and Chicago[3] studies, has ample numbers of subjects. Another explanation, suggested by Graff-Radford and colleagues,[11] may be that these differences can be attributed in part to an age effect. Although the patients in the study by Graff-Radford et al were, on average, younger than the Indianapolis subjects, this is unlikely to explain the differences between our results and those obtained from New York and Chicago because the ages of those cohorts were similar.

Another possible explanation is population stratification. The *APOE* ε4 allele frequency in normal Indianapolis subjects is 17.6%, a value that is now significantly lower than the 21.7% observed in normal elderly Yoruba[15] (*P*=.049). However, the ε2 allele frequencies in normal subjects were practically the same: 11.5% compared with 11.1% in the Yoruba.[15] The ε4 frequency among African Americans from Indianapolis is now similar to the frequency found in African American controls living in cities in the southeastern United States (frequency, 18.1%),[11] whereas the frequency of the ε4 allele was significantly higher in African American controls from Manhattan, NY (20.1%),[14] and the whole African American cohort from Chicago (20.9%)[3] (genotype distribution was not published for the controls). These frequencies were similar to the ones observed in the Yoruba and Jamaicans (22.0%).[24] Taken together, these differences could be the result of varying degrees of admixture of Africans with other populations, eg, whites or American Indians at different study sites. It is difficult to determine the extent of admixture demographically, but analyses of single nucleotide polymorphisms combined with haplotype analyses may provide a clearer picture. We intend to undertake these studies with our 2 cohorts. Although we are aware of the complexity of racial classification, especially with regard to admixture, the observation that *APOE* ε4 bears differential associations with AD in African Americans and in African Yoruba, despite their presumed racial similarity, is worth pursuing.

Also, these differences in ε4-associated AD risk could be due to different environmental influences and interactions with genetic factors. For example, dietary intake varies between African Americans and the Yoruba; the Yoruba consume foods low in calories and fat and high in fiber, whereas the African American diet is high in fat and sodium and low in fiber. This dietary difference is evident in the lower levels of cholesterol and the higher levels of vitamin B_{12} seen in the Yoruba compared with the Indianapolis subjects.[25] Not surprisingly, the Yoruba also have a lower incidence of vascular disease and vascular risk factors.[25] We hope that by continuing to explore genetic and environmental factors and their interactions in the Yoruba and African Americans, we can establish a disease model for AD that can account for the differences in risk for AD between these 2 populations.

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Table 1

Baseline Characteristics and *APOE* Allele and Genotype Frequencies by Diagnosis

	Subjects With AD (n = 162)	Normal Subjects (n = 318)
Demographic characteristics		
Age, mean ± SD, y	83.8 ± 6.4	78.8 ± 6.6
Age in quantiles, y		
Minimum	70.5	66.9
25th	79.6	73.4
50th	83.2	77.9
75th	88.1	82.3
Maximum	102.6	98.8
No. (%) female	103 (63.6)	209 (65.7)
Highest level of education, mean ± SD, y	8.7 ± 3.7	9.9 ± 3.0
Follow-up, mean ± SD, y	3.6 ± 3.5	3.8 ± 3.4
Allele frequency, No. (%)		
ε2	20 (6.2)	73 (11.5)
ε3	195 (60.2)	451 (70.9)
ε4	109 (33.6)	112 (17.6)
Genotype frequency, No. (%)		
ε2/ε2	1 (0.6)	6 (1.9)
ε2/ε3	12 (7.4)	48 (15.1)
ε2/ε4	6 (3.7)	13 (4.1)
ε3/ε3	61 (37.7)	163 (51.3)
ε3/ε4	61 (37.7)	77 (24.2)
ε4/ε4	21 (13.0)	11 (3.5)

Abbreviations: AD, Alzheimer disease; *APOE*, apolipoprotein E.

Table 2

Logistic Regression Results on Subjects With AD vs Normal Subjects by *APOE* Genotype

	OR (95% CI)	*P* Value
Sex, male vs female	1.22 (0.77–1.92)	.41
Age	1.14 (1.10–1.18)	<.001
Highest level of education	0.90 (0.85–0.96)	.002
APOE genotype		
ε2/ε2, ε2/ε3 vs ε3/ε3	0.42 (0.20–0.89)	.02
ε2/ε4 vs ε3/ε3	1.34 (0.44–4.13)	.61
ε3/ε4 vs ε3/ε3	2.32 (1.41–3.82)	<.001
ε4/ε4 vs ε3/ε3	7.19 (3.00–17.29)	<.001

Abbreviations: AD, Alzheimer disease; *APOE*, apolipoprotein E; CI, confidence interval; OR, odds ratio.