

Published in final edited form as:

Ann Neurol. 2006 January ; 59(1): 182–185. doi:10.1002/ana.20694.

## APOE $\epsilon$ 4 Is Not Associated with Alzheimer's Disease in Elderly Nigerians

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### Abstract

Since 1992, research teams from Indiana University and the University of Ibadan have been collecting and comparing data from two diverse, elderly populations to identify risk factors for dementia and Alzheimer's disease. *Apolipoprotein E (APOE)* was genotyped in 2,245 Nigerian samples. Of these, 830 had a diagnosis: 459 were normal, and 140 had dementia including 123 diagnosed with Alzheimer's disease. In contrast with other populations, the *APOE*  $\epsilon$ 4 allele was not significantly associated with Alzheimer's disease or dementia. This lack of association in the Yoruba might reflect genetic variation, environmental factors, as well as genetic/environmental interactions.

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Alzheimer's disease (AD) is the most common form of dementia, accounting for a major part of public health spending in many developed societies<sup>1</sup> and becoming an economic burden in developing countries. <sup>2</sup> A meta-analysis including data from several ethnic groups showed that the  $\epsilon$ 4 allele of *apolipoprotein E* gene (*APOE*) constitutes a major susceptibility factor for the development of AD.<sup>3</sup> However, this relation appears less consistent among African American<sup>4,5</sup> and Hispanic<sup>4</sup> populations.

We have previously published our prevalence findings on the relation between *APOE* and the risk for AD in the Indianapolis-Ibadan Dementia Study, a longitudinal, cross-cultural study of AD in African Americans in Indianapolis and the Yoruba in Ibadan, Nigeria.<sup>6,7</sup> Among African Americans, a significant association between  $\epsilon$ 4 and AD was observed, with the association being dose dependent.<sup>6</sup> In the Yoruba, neither one nor two  $\epsilon$ 4 alleles were associated with an increased risk for AD. However, only 12 Yoruba subjects had AD among the 56 samples that were genotyped.<sup>7</sup>

Since this report was published, we have *APOE* genotypes on 2,245 Ibadan subjects and clinical assessments on 830, including 123 with AD. Our results are presented here.

## Subjects and Methods

### Study Design and Subjects

Data are derived from a population-based study on the prevalence and incidence of AD and dementia in Yoruba 65 years or older ( $n = 4,425$ ). The original cohort of 2,212 was enriched by an additional prevalence wave in 2001. A detailed description of the study methodology has been published previously.<sup>8</sup> Institutional review boards at both the University of Ibadan and Indiana University have approved the study protocol. Only individuals who provided signed informed consent were studied. Data were collected from a baseline wave conducted in 1992 and 1993 and follow-up evaluations conducted 2, 5, and 8 years after baseline. At each wave, a two-phase design was used. In the first phase, subjects were interviewed in their homes using the Community Screening Instrument for Dementia.<sup>9</sup> Based on the screening performance, selected participants received a full diagnostic workup in the second phase.

At each follow-up wave, study participants were divided into three performance groups: good, intermediate, and poor. Group division was based on the subjects' current screening scores. In addition, change scores from previous waves were calculated. Subjects were also categorized into poor, intermediate, and good change groups. Cutoff points on change scores were derived so that approximately 5% of subjects with the worst change scores (most declines) were in the poor change group and approximately 8% of subjects with the next worst change scores were in the intermediate change group. The cross-sectional and longitudinal groupings were combined into one in which subjects were categorized by the worst of the two. All subjects falling into the poor performance group were chosen for clinical assessment to ensure that participants with the greatest probability of having dementia would be diagnosed. Participants were randomly sampled from the intermediate performance group until 50% had clinical assessments and from the good performance group (weighted for 75% of age 75 years and older) until 5% had clinical assessments.

Clinical evaluations consisted of an informant interview, neuropsychological testing, and examination by a physician. Diagnoses of normal, cognitive impairment, or dementia were made by consensus of study physicians and neuropsychologists from both sites. For a diagnosis of dementia, both *International Classification of Diseases, 10th Revision (ICD-10)*<sup>10</sup> and *Diagnostic and Statistical Manual of Mental Disorders, Revised Third Edition (DSM-III-R)*<sup>11</sup> criteria had to be met. The National Institute for Neurological and Communicative Diseases and Stroke-Alzheimer Disease and Related Disorders Association criteria were used for diagnosis of probable and possible AD.<sup>12</sup>

### Apolipoprotein E Analyses

DNA was extracted from blood spots collected on filter paper<sup>7</sup> and from fresh blood using standard protocols. *APOE* genotypes were determined by *HhaI* digestion.<sup>13</sup>

### Statistical Analyses

Only subjects who had an *APOE* genotype and a clinical evaluation were included in this study. The demented or AD group consists of subjects who were diagnosed with dementia or AD at any time throughout the course of the study. Of the remaining subjects evaluated clinically, subjects were classified according to the diagnosis of their most recent clinical assessment. Those who were diagnosed as normal at their most recent clinical assessment were considered normal. Subjects whose most recent diagnosis was cognitive impairment were excluded from this analysis. Demographic characteristics and *APOE* allele frequencies were compared with

*t* tests for continuous variables and  $\chi^2$  tests for categorical variables. Logistic regression models adjusting for age at diagnosis, gender, and formal education (any vs none) were used to calculate the odds ratios and 95% confidence intervals for AD and dementia for various *APOE* genotypes, using the  $\epsilon 3/\epsilon 3$  genotype as the reference group. *p* values less than 0.05 were considered statistically significant.

## Results

A total of 2,245 DNA samples were genotyped for *APOE*. Of these samples, 830 participants had a clinical diagnosis. No significant differences were seen in the frequency of the *APOE* genotypes between the clinically evaluated subjects and those without a clinical evaluation ( $p = 0.3898$ ). However, the subjects with a clinical diagnosis are significantly older ( $73.9 \pm 8.0$  vs  $73.0 \pm 5.7$ ;  $p = 0.0054$ ), more likely to be female (71.8% vs 62.9%;  $p < 0.0001$ ), and had no formal education (87.4% vs 84.2%;  $p = 0.0391$ ). These differences were expected because there is oversampling of the poor performance group.

Of the 830 clinically evaluated subjects, 459 were normal and 140 were diagnosed with dementia (38 subjects at prevalence and 102 at incidence), of which 123 were diagnosed with AD (30 subjects at prevalence and 93 at incidence). The remaining 231 subjects were diagnosed with cognitive impairment and left out of the analyses. Table 1 shows the baseline characteristics and *APOE* genotype and allele frequencies for subjects with each diagnosis. There was a significant difference in gender between both the demented and AD groups and normal subjects (demented vs normal:  $p = 0.0017$ ; AD vs normal:  $p < 0.0001$ ). Subjects with dementia and AD were significantly older than normal subjects ( $p < 0.0001$  for both comparisons). In addition, the demented and AD groups contained less subjects who attended school ( $p = 0.0295$  and  $0.0075$ , respectively). All subjects were followed for similar amounts of time (demented vs normal:  $p = 0.1025$ ; AD vs normal:  $p = 0.0637$ ). Follow-up times for those with and without  $\epsilon 4$  were similar (5.4 vs 5.2 years, respectively).

None of the *APOE* alleles were significantly increased in the AD ( $\epsilon 2$ :  $p = 0.6717$ ;  $\epsilon 3$ :  $p = 0.3171$ ;  $\epsilon 4$ :  $p = 0.1484$ ) or dementia ( $\epsilon 2$ :  $p = 0.4878$ ;  $\epsilon 3$ :  $p = 0.7226$ ;  $\epsilon 4$ :  $p = 0.3586$ ) groups compared with normal subjects. There were no significant differences in the distributions of the number of  $\epsilon 4$  alleles between the AD ( $p = 0.3570$ ) or demented ( $p = 0.6578$ ) subjects and normal subjects.

Logistic regression results of the association of *APOE* with AD or dementia, after adjusting for gender, age at diagnosis, and education, are shown in Table 2. Again, there were no significant differences in the number of subjects with one or two copies of  $\epsilon 4$  among the groups. In addition, the  $\epsilon 2$  allele did not confer protection against the risk for dementia or AD. Gender and age at diagnosis were significant factors.

## Discussion

In our analysis of 123 patients with AD and 140 patients with dementia, there was no relation between *APOE*  $\epsilon 4$  and AD or dementia in the Yoruba. These results using data from all the prevalence and incidence waves of our study are consistent with our earlier observation that  $\epsilon 4$  is not a significant risk factor for AD in Nigerians.<sup>7</sup> The results were similar if the prevalence and incident cases were analyzed separately. The strength of using incident cases is that case accrual is unlikely to have been affected by differential mortality between AD cases and healthy subjects and between genotype groups within AD. As shown in a previous article,<sup>13</sup> we did not find an association between  $\epsilon 4$  and mortality risk. However, having dementia significantly increased the risk for mortality.<sup>14</sup>

The lack of association contrasts with our previous findings of increased risk for AD with  $\epsilon 4/\epsilon 4$  in African Americans.<sup>6</sup> It is possible that the Yoruba  $\epsilon 4$  carriers are dying earlier of other diseases, such as, cardiovascular disease. However, the  $\epsilon 4$  allele frequency is not significantly different in the two cohorts (0.217 vs 0.218).<sup>6</sup> Interestingly, the Yoruba have a lower incidence of both vascular disease and vascular risk factors including hypertension than did the African Americans.<sup>15</sup> Also, cholesterol and lipid levels are much lower in the Yoruba.

This lack of association together with the low incidence rate of AD makes the Yoruba an interesting population to study,<sup>7-8</sup> especially in contrast with the African Americans. Both genetic and environmental factors may be responsible. There may be more variation within the African genome.<sup>16</sup> We do not know how similar these two cohorts are genetically. The increased incidence of AD and the association with  $\epsilon 4$  in the African Americans could be due to admixture. In addition, environmental risk factors that may play a role in AD (ie, high-fat diet and vascular disease) are not as common in the Yoruba. We are continuing to explore these possibilities in these two cohorts.

## Acknowledgments

This research was supported by NIH (National Institute of Aging, RO1 AG09956, O.G., A.O., D.B., B.F., F.W.U., R.M.E., V.S.G., K.A.L., S.G., K.A.H., M.C.H., J.R.M.; P30 AG10133, F.W.U., R.M.E., K.A.L., S.G., H.C.H., J.R.M.).

We acknowledge the Ibadan community for their support and cooperation.

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**Table 1**

Baseline Characteristics and APOE Allele and Genotype Frequencies by Diagnosis

Characteristic	Dementia (n = 140)	AD (n = 123)	Normals (n = 459)
Demographics			
Age at diagnosis (mean $\pm$ SD)	82.4 $\pm$ 9.3	82.8 $\pm$ 9.4	77.8 $\pm$ 7.5
Female subjects, N (%)	112 (80.0%)	104 (84.6%)	303 (66.0%)
Had formal education	12 (8.6%)	8 (6.5%)	73 (15.9%)
Years of follow-up (mean $\pm$ SD)	5.5 $\pm$ 3.5	5.6 $\pm$ 3.4	4.9 $\pm$ 3.8
Allele			
$\epsilon$ 2	27 (9.6%)	25 (10.2%)	102 (11.1%)
$\epsilon$ 3	185 (66.1%)	157 (63.8%)	617 (67.2%)
$\epsilon$ 4	68 (24.3%)	64 (26.0%)	199 (21.7%)
Genotype			
$\epsilon$ 2/ $\epsilon$ 2	0 (0.0%)	0 (0.0%)	4 (0.9%)
$\epsilon$ 2/ $\epsilon$ 3	22 (15.7%)	20 (16.3%)	73 (15.9%)
$\epsilon$ 2/ $\epsilon$ 4	5 (3.6%)	5 (4.1%)	21 (4.6%)
$\epsilon$ 3/ $\epsilon$ 3	59 (42.1%)	48 (39.0%)	206 (44.9%)
$\epsilon$ 3/ $\epsilon$ 4	45 (32.1%)	41 (33.3%)	132 (28.8%)
$\epsilon$ 4/ $\epsilon$ 4	9 (6.4%)	9 (7.3%)	23 (5.0%)

AD = Alzheimer's disease; SD = standard deviation.

**Table 2**  
 Logistic Regression Results on Dementia versus Normal Subjects and AD versus Normal Subjects by APOE genotype

Measure	Dementia			AD		
	OR	95% CI	<i>P</i>	OR	95% CI	<i>P</i>
Sex: M vs F	0.44	0.27–0.74	0.0016	0.32	0.17–0.57	0.0001
Age at diagnosis	1.08	1.05–1.10	<0.0001	1.09	1.06–1.12	<0.0001
Attended school	0.59	0.29–1.18	0.1359	0.48	0.21–1.11	0.0862
<i>APOE</i> : 22/23 vs 33	1.21	0.68–2.17	0.5186	1.44	0.77–2.69	0.2514
<i>APOE</i> : 24/34 vs 33	1.22	0.77–1.91	0.4000	1.38	0.85–2.25	0.1950
<i>APOE</i> : 44 vs 33	1.60	0.67–3.82	0.2897	2.13	0.86–5.23	0.1007

AD = Alzheimer's disease; OR = odds ratio; CI = confidence interval.