Serum cholesterol, *APOE* genotype, and the risk of Alzheimer's disease: A population-based study of African Americans

Article abstract—A significant interaction among total serum cholesterol (TC), *APOE* genotype, and AD risk was found in a population-based study of elderly African Americans. Increasing TC was associated with increased AD risk in the group with no ϵ4 alleles, whereas TC was not associated with increased AD risk in the group with one or more ϵ4 alleles. Further study of the relationship between cholesterol and *APOE* genotype is needed to confirm this association, but the results suggest that cholesterol may be a potentially modifiable environmental risk factor for AD. **Key words:** Cholesterol—*APOE* genotype—AD—African American.

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The *APOE* ϵ4 allele has been established as a dose-dependent risk factor for the development of AD in most populations.[1] The role of the ϵ4 allele in AD risk is not as clear in African Americans, in whom its effect appears to be weaker and perhaps confined only to the homozygous state.[2,3]

The *APOE* system also is known to play an integral part in cholesterol transport to the neuron. A few clinical reports have suggested an interaction among serum cholesterol, *APOE* genotype, and AD.[4,5] In the current study, the interaction between total serum cholesterol (TC) and *APOE* genotype for risk of AD was evaluated in a population-based cohort of elderly African Americans (AA) who are part of the Indianapolis-Ibadan dementia project.[6]

Methods. The cohort consisted of African Americans older than 65 years from 29 contiguous census tracts in Indianapolis, IN, where African Americans represented 80% of the population in the 1990 US Census Report. Door-to-door random sampling of residential addresses yielded 2,582 eligible persons, 2,212 of whom were included as participants. Distributions of age, gender, and socioeconomic status were representative of all African Americans in Indianapolis and Indiana.

The Community Screening Interview for Dementia (CSI-D) was used to screen individuals and classify them into performance groups.[7] Random sampling within performance groups with oversampling of the poor performers was used to select individuals for in-depth clinical assessment.

For the diagnosis of dementia, both *Diagnostic and Statistical Manual for Mental Disorders,* third edition revised (DSM-III-R) and International Classification of Diseases, 10th edition (ICD-10) criteria had to be met. Both probable and possible AD were diagnosed by National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) criteria. Vascular and secondary dementias were diagnosed by ICD-10 criteria.

Clinical assessments were performed on 524 individuals selected at both prevalence and incidence waves by CSI-D scores. Of these, 411 individuals were assessed as AD or normal, and 87 as having both the *APOE* genotype and TC. The group analyzed was not significantly different in mean age, education level, or gender distribution from the larger sample of AD and normal individuals. Of the 87 individuals, none were on cholesterol-lowering agents; 2 (one AD) were on estrogen replacement, and 5 (2 AD) were on insulin or oral hypoglycemics.

APOE genotyping. For *APOE* analyses, DNA eluted from dried blood spots or DNA prepared from liquid blood samples was used. As described previously, *APOE* genotypes were determined by Hhal digestion of the amplified product.[8,9]

Cholesterol determinations. An accredited laboratory analyzed the cholesterol values using automated equipment. There was an average of 4.5 months between clinical assessment and TC determination.

Statistical analysis. Demographic factors were compared for normal versus AD subjects using *t*-tests for continuous variables and Fisher's exact tests for categorical variables. The logistic regression model included age and TC as continuous variables (TC centered about 0 by subtraction of the sample mean), *APOE*, and TC–*APOE* interaction as independent variables. Because *APOE* 4/4 numbers were small (AD 4/4 = 9, 3/4 = 15, 2/4 = 0; normal 4/4 = 1, 3/4 = 15, 2/4 = 1), homozygous and heterozygous ϵ4 groups were merged. The odds ratios (ORs) for developing AD with increasing cholesterol, for the ϵ4 and no ϵ4 groups, were calculated from the logistic regression model. The ORs were tested for significance by calculating Wald's chi-square statistics and *p* values using logistic parameter estimates and the variance–covariance matrix of these parameter estimates.

Results. The AD and normal groups did not differ significantly with respect to gender or education (table 1). The AD group was older than the normal group ($p < 0.01$) for both ϵ4 and no ϵ4 subgroups. Mean TC in the ϵ4 group was similar for both AD and normal individuals (see table 1). In the no ϵ4 group, mean TC was higher in those with AD than in normals.

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Table 1 *Demographics, cholesterol, and self-reported vascular history for AD versus normal subjects by genotype*

	AD		Normal	
	ε4, n = 24	No ε4, n = 22	ε4, n = 17	No ε4, n = 24
Gender: % female	54.2	63.6	52.9	50.0
Years of education, mean (SD)	8.9 (3.7)	8.1 (2.8)	7.8 (2.7)	9.4 (2.9)
Age, y, mean (SD)	81.8 (5.6)*	82.0 (6.9)†	77.5 (7.2)*	77.8 (7.1)†
Cholesterol, mean (SD)	224 (45.7)	230.0 (50.7)†	229 (50.1)	202 (40.8)†
High cholesterol, % >240 mg/dL	33.3	36.4	35.3	16.7
Diabetes	19.1	10.5	17.7	8.3
Heart attack or angina	23.8	15.8	41.2	33.3
Hypertension	47.6	47.4	58.8	54.2
Stroke	14.3	10.5	17.7	16.7

* Significant difference between normal and AD for the ε4 group.
† Significant difference between normal and AD for the no ε4 group.

Significant difference defined as $p < 0.05$.

Logistic regression results showed a significant cholesterol–genotype interaction ($p = 0.04$) after adjustment for age (table 2 and figure). In the no ϵ4 group, increasing cholesterol was associated with increasing risk for AD (OR = 1.018; $p = 0.027$). With each 20-point increase in TC in the no ϵ4 group, the OR for AD was 1.42. In contrast, there was no significant association between TC and AD risk (OR = 0.996; $p = 0.551$) in the ϵ4 group.

Discussion. In this study of elderly African Americans, a significant interaction between serum total cholesterol (TC), *APOE* ϵ4, and AD risk was found. In the group of individuals with no ϵ4 allele, increasing cholesterol levels were significantly associated with an increasing risk of AD. In the group of individuals with an ϵ4 allele, the risk of AD was not associated with cholesterol level.

There have been two similar previously published clinical studies. Although the methods of these two studies are not directly comparable with those of our study, the findings tend to support our conclusions. In a longitudinal population-based study of Finnish men, Notkola et al.[4] reported that high cholesterol was a significant predictor for the development of AD after adjustment for age and ϵ4.

Jarvik et al.[5] examined *APOE* genotype, TC, and AD risk in a community-based study of 206 AD cases and 276 controls, with a mean age of 79 years. A significant interaction was found between *APOE* and TC, with the relationship of *APOE* and AD at least partially dependent on TC, as well as on age and gender. This study focused on the effect of *APOE* while controlling for TC, whereas our study focused on the effect of TC while controlling for *APOE*.

Our study has several limitations. Random, as opposed to fasting, cholesterol levels were obtained. Nonfasting values and randomly obtained levels of TC are not significantly different from fasting levels for an individual.[10] Any variation in levels caused by these variables would be equally distributed between all subjects, and would not be expected to change the interaction found. It has been shown that TC may decline with increasing age or before the onset of AD.[4] In our study, TC was obtained near the time of AD diagnosis and may have been higher before disease onset. If TC had been collected before dementia diagnosis, the association between cholesterol and AD in both groups might have been stronger.

Table 2 *Logistic regression results for AD versus normal individuals*

	Parameter estimate	Standard error	Odds ratio	*p* Value
Age	0.114	0.039	1.121	0.003
Cholesterol	0.018	0.008	1.018	0.027
Genotype (ε4 vs no ε4)	0.387	0.476	1.473	0.415
Cholesterol* genotype	−0.022	0.011	0.978	0.042

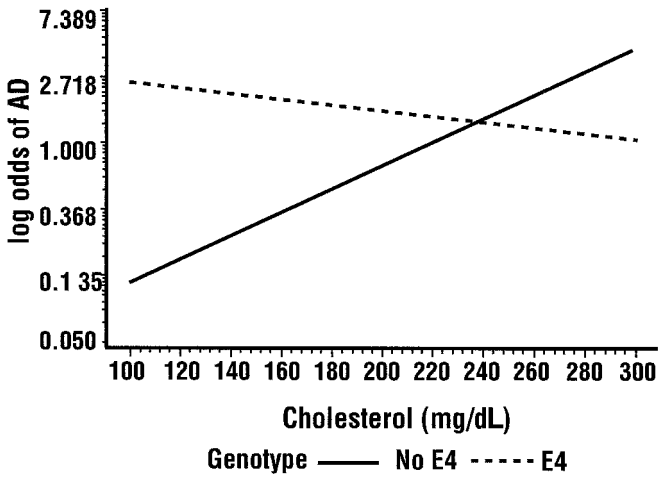

Figure. Lot of log odds of AD versus cholesterol level (mg/dL) by ϵ4 status.

In the current study, *APOE* genotyping and cholesterol values were available for only a small group (20%) of all individuals diagnosed as AD or normal. This was partly because in the initial stages of the study, blood samples were obtained only on individuals deemed to have presumptive dementia. Later in the study, blood samples were requested from all clinically assessed subjects. However, the individuals tested did not differ significantly in mean age or years of education from the individuals not tested. The current study was cross-sectional in design. In the future, we intend periodically to evaluate lipid levels in all nondemented individuals to clarify further the relationship among *APOE*, cholesterol, and the subsequent development of AD.

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Absence of cystatin C mutation in sporadic cerebral amyloid angiopathy–related hemorrhage

Article abstract—In Icelandic pedigrees a cystatin C mutation, glutamine 68 (L68Q), causes autosomal dominant cerebral amyloid angiopathy–related hemorrhage (CAAH). We examined 33 patients with sporadic CAAH for this mutation. None carried L68Q and, including this report, only one of 52 published cases of sporadic CAAH has had the cystatin C mutation. Despite vascular colocalization of cystatin C with amyloid β-protein, cystatin C L68Q is rare in sporadic CAAH. **Key words:** Cerebral amyloid angiopathy—Intracerebral hemorrhage—Cystatin C.

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Intracerebral hemorrhage (ICH) causes 30% to 50% mortality and significant disability. Although hypertension is the most frequent known cause of ICH, cerebral amyloid angiopathy–related hemorrhage (CAAH) accounts for 5% to 10% of primary nontraumatic ICH. Three genes have been implicated in CAAH. Several series have confirmed that apolipoprotein E (*APOE* for gene; apoE for protein) predisposes to the sporadic form of CAAH[1,2]; current evidence suggests that the *APOE* ϵ4 allele increases amyloid β-protein deposition in the cerebral vasculature[1] whereas the ϵ2 allele leads to rupture of amyloid-laden blood vessels,[2] the latter genetic association being specific to this form of ICH. These polymorphisms do not, however, account for all cases of CAAH as many of the patients with this form of ICH have the ϵ3/ϵ3 genotype.[2] Genetic mutations in amyloid precursor protein (*APP*) have been documented in the Dutch (*APP 693*) and Flemish (*APP 692*) types of hereditary cerebral hemorrhage with amyloidosis. A mutation in cystatin C is recognized in Icelandic pedigrees as the cause of hereditary cystatin C amy-

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