Apolipoprotein E Polymorphism and Alzheimer Disease

The Indo-US Cross-National Dementia Study

Mary Ganguli, MD, MPH; Vijay Chandra, MD, PhD; M. Ilyas Kamboh, PhD; Janet M. Johnston, PhD; Hiroko H. Dodge, PhD; B. K. Thelma, PhD; Ramesh C. Juyal, PhD; Rajesh Pandav, MBBS; Steven H. Belle, PhD; Steven T. DeKosky, MD

Background: The APOE*E4 allele of the gene for apolipoprotein E (APOE) has been reported as a risk factor for Alzheimer disease (AD) to varying degrees in different ethnic groups.

Objective: To compare APOE*E4-AD epidemiological associations in India and the United States in a cross-national epidemiological study.

Design: Case-control design within 2 cohort studies, using standardized cognitive screening and clinical evaluation to identify AD and other dementias and polymerase chain reaction to identify APOE genotyping.

Participants: Rural community samples, aged 55 years or older (n=4450) in Ballabgarh, India, and 70 years or older (n=886) in the Monongahela Valley region of southwestern Pennsylvania.

Main Outcome Measures: Criteria of the National Institute of Neurological and Communicative Disorders and Stroke–Alzheimer’s Disease and Related Disorders Association for probable and possible AD and Clinical Dementia Rating (CDR) scale for dementia staging.

Results: Frequency of APOE*E4 was significantly lower (P<.001) in Ballabgarh vs the Monongahela Valley (0.07 vs 0.11). Frequency of probable or possible AD, with CDR of at least 1.0, in the Indian vs US samples, was as follows: aged 55 to 69 years, 0.1% (Indian sample only); aged 70 to 79 years, 0.7% vs 3.1%; aged 80 years or older, 4.0% vs 15.7%. Among those aged 70 years or older, adjusted odds ratios (95% confidence interval) for AD among carriers of APOE*E4 vs noncarriers were 3.4 (1.2-9.3) and 2.3 (1.3-4.0) in the Indian and US samples, respectively, and not significantly different between cohorts (P=.20).

Conclusion: This first report of APOE*E4 and AD from the Indian subcontinent shows very low prevalence of AD in Ballabgarh, India, but association of APOE*E4 with AD at similar strength in Indian and US samples.

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Dementia affects 5% to 10% of individuals aged 65 years or older in Europe and North America at any given time. Alzheimer disease (AD) is the most frequent cause of dementia.1 Of the many putative genetic risk factors for AD, only the gene for apolipoprotein E (APOE) has thus far been shown to be associated with both early- and late-onset AD of sporadic and familial varieties.2 The *E4 allele of the APOE gene has been consistently shown to be associated with AD in many studies of white populations, whereas the *E2 allele has in some studies appeared to be protective against AD.3 Findings appear similar in a Chinese population but, based on a number of studies in African, African American, and Hispanic populations, the evidence of an APOE-AD association is mixed.4-8 Thus, there may be interactions between APOE and racial or ethnic group as well as environmental factors that might modify the increased risk of AD conferred by the APOE*E4 allele in different populations.7 To our knowledge, there are no previous reports from the Indian subcontinent, of which the population represents approximately one sixth that of the world.9 Under a National Institute on Ageing-sponsored program of cross-national investigations,10 the Indo-US Cross-National Dementia Epidemiology Study was funded as a collaborative venture between the University of Pittsburgh, Pittsburgh, Pa, and the Centre for Ageing Research in India (CARI), New Delhi. The study was designed to compare the prevalence, incidence, risk factors, and outcome of AD and other...
dementias between the rural communities of Ballabgarh in northern India and the Monongahela Valley region in southwestern Pennsylvania.

The prevalence of AD and other dementias among the elderly in Ballabgarh was recently reported to be the lowest thus far reported in the world, suggesting that different risk and protective factors might be operating in this Indian community than in other populations. We herein report and compare the relationship between AD and the APOE polymorphism in large, community-based samples of the elderly populations of Ballabgarh and the Monongahela Valley, to answer the following 3 primary research questions: (1) Is the frequency of the APOE*E4 allele different between study cohorts from both populations? (2) Is the APOE*E4 allele associated with AD in both cohorts? (3) If so, is the strength of the association similar in the two cohorts?

When the APOE gene was first reported as a risk factor for AD in 1993, the MoVIES cohort was in its third biennial data collection wave. From January 26, 1994, through October 23, 1997, blood specimens for APOE genotyping were collected from surviving members of the MoVIES cohort, who by that time were all older than 70 years. Of the 1681 subjects in the original (1987-1989) MoVIES cohort, 886 white individuals who were still alive and participating in the study and had not moved to nursing homes underwent blood sample draws for genotyping. The likely survival bias in the genotyped subsample of the MoVIES cohort precludes its use for prevalence estimation but does not affect its usefulness for case-control and other risk and protective factor studies.

Blood samples were collected by venipuncture (88%) or by finger- or earlobe-stick (12%). The DNA was isolated with the use of a commercially available kit (QIAamp kit; QIAGEN Inc, Chatsworth, Calif). The APOE genotyping was performed by means of a polymerase chain reaction protocol as described previously.

**BALLABGARH STUDY SITE**

Ballabgarh is a rural agricultural area in the state of Haryana in northern India. The initial phase of the study was devoted to developing appropriate assessment tools for the Hindi-speaking Ballabgarh elderly population, approximately three quarters of whom were totally illiterate. The development of the screening battery,22-23 the functional ability scale,24 and the clinical diagnostic protocol used in our study and designed to maximize comparability to the MoVIES study have been described in detail elsewhere.

After instrument development, the entire population aged 55 years or older in the 28 villages in the Ballabgarh study area was recruited and surveyed from December 4, 1995, through December 9, 1997. Recruitment for the present study was extended to all adults aged 55 years or older, rather than 65, because of the relatively short life expectancy (62.1 years at birth) of individuals in this area. Demographic data collected included age confirmation (reported previously), education (years of formal schooling), and literacy (ability to write a sentence and read the local language). The prevalence of AD and other dementias among the elderly in Ballabgarh was recently reported to be the lowest thus far reported in the world, suggesting that different risk and protective factors might be operating in this Indian community than in other populations. We herein report and compare the relationship between AD and the APOE polymorphism in large, community-based samples of the elderly populations of Ballabgarh and the Monongahela Valley, to answer the following 3 primary research questions: (1) Is the frequency of the APOE*E4 allele different between study cohorts from both populations? (2) Is the APOE*E4 allele associated with AD in both cohorts? (3) If so, is the strength of the association similar in the two cohorts?

**RESULTS**

**DEMOGRAPHICS**

As of the reference dates, the MoVIES cohort (n=886) had a mean age of 79.3 (4.8) years, with a median of 78.7 years; 61.1% were aged 70 to 79 years, and 38.9% were aged 80 years or older. Women constituted 64.2% of the sample. For education, 38.3% did not graduate from high school, 36.0% were high school graduates, and 25.7% had more than a high school education.

In the Ballabgarh cohort (n=4450), the mean age was 67.1 (7.2) years, with a median of 65.7 years as of the reference dates; 72.0% were aged 55 to 69 years, 21.8% were aged 70 to 79 years, and 6.2% were aged 80 years or older. Women constituted 64.2% of the sample. Although 73.9% had no formal schooling, 1.6% of unschooled individuals had some literacy as defined ear-
newspaper). Using methods and criteria closely following those in the MoVIES project, all participants underwent cognitive screening. Those whose scores fell below operational criteria for cognitive impairment and a random sample of unimpaired controls were selected for detailed clinical evaluation. The standardized clinical evaluation protocol, also following that of the MoVIES project, culminated in a DSM-III-R diagnosis of dementia syndrome, a diagnosis of probable or possible AD according to NINCDS-ADRDA criteria, and a CDR rating of the stage of dementia. Further descriptions of the cohort and of screening and diagnostic methods are found in previous reports.11,22-25

Blood sample draws for APOE genotyping were performed during the prevalence (baseline) phase. Of the 5126 subjects originally recruited in this cohort, 4450 individuals (86.8% of the cohort) provided finger-stick blood samples. The DNA was isolated with the use of the blood kits described above, and APOE genotyping was performed by means of polymerase chain reaction analysis. Reliability was established between both laboratories by blind genotyping in the Indian laboratory of a sample of specimens provided from the US laboratory, and by blind reading in the US laboratory of a sample of gel photographs prepared in the Indian laboratory. Government of India regulations prohibit the export of human specimens to overseas laboratories for research.

At both study sites, informed consent was obtained after full explanation of the nature of the study procedures, all of which were performed according to protocols approved by the University of Pittsburgh Institutional Review Board. For the Ballabgarh site, approval was also obtained from the Human Volunteers Protection Committee of CARI.

STATISTICAL ANALYSES

Means (SDs) and medians were calculated for age in both cohorts. The Monongahela Valley cohort was classified into groups aged 70 to 79 years and 80 years or older, and the Ballabgarh cohort was classified into groups aged 55 to 69 years, 70 to 79 years, and 80 years or older. Cross-national comparisons used only the groups aged 70 to 79 years and 80 years or older. Proportions of each cohort by sex and education or literacy level were calculated. For the present analyses, we classified the Ballabgarh cohort according to literacy (rather than education) as 26.5% literate and 73.5% illiterate.

Frequencies of APOE alleles in both cohorts are presented in Table 1.

In the MoVIES cohort, the frequencies of the APOE*E2, APOE*E3, and APOE*E4 alleles were 0.07, 0.82, and 0.11, respectively; the genotype distributions were in Hardy-Weinberg equilibrium (P = .76). Of 186 APOE*E4 carriers, 11 were *E4/*E4 homozygous. The APOE*E4 frequencies in both age groups (Table 1) were not significantly different (P = .48).

In the Ballabgarh cohort, the frequencies of the APOE*E2, APOE*E3, and APOE*E4 alleles were 0.04, 0.89, and 0.07, respectively. Of 632 APOE*E4 carriers, 22 were *E4/*E4 homozygous. The distribution of APOE genotypes in the Ballabgarh cohort deviated from Hardy-Weinberg equilibrium (χ²), was 9.12 compared with the critical value of 7.81; P = .03). Since equilibrium may not hold for the population after the age of reproduction, this deviation in our older population might have been the result of selective survival. Further, rapid declines in infant and child mortality in recent decades may have rendered the population unstable with regard to APOE frequencies.

The APOE*E4 frequency in the 3 age groups (Table 1) showed no trend with age in Ballabgarh. The frequencies of APOE*E2 and APOE*E4 alleles in the population aged 70 years or older were significantly lower in the Ballabgarh than in the MoVIES cohort (P < .001 by χ²).

FREQUENCY OF AD AND OTHER DEMENTIAS

In the MoVIES cohort, 132 subjects (14.9%) had any or all dementia according to DSM-III-R and a CDR stage of at least 0.5 as of the reference date. Of those with any
dementia, 115 (87.1%) had probable or possible AD according to NINCDS-ADRDA criteria. Restricting the analyses to subjects with CDR stage of at least 1.0, 75 subjects (8.5%) had any dementia, 71 (94.7%) of whom had probable or possible AD.

In the Ballabgarh cohort, 36 (0.8%) had any dementia according to DSM-III-R and a CDR stage of at least 0.5. Of the demented, 28 (77.8%) had probable or possible AD according to NINCDS-ADRDA criteria. Among those with CDR stage of at least 1.0, there were 29 (0.7%) subjects with any dementia, 21 (72.4%) of whom had probable or possible AD. Further breakdown of these diagnosis and stage categories in both samples by age groups is shown in Table 1, and by genotype in Table 2.

### ASSOCIATIONS BETWEEN AD AND APOE GENOTYPES

Adjusted ORs used to estimate cross-sectional associations between APOE genotypes, and AD and dementia in both cohorts are shown in Table 3.

#### APOE*E4 ALLELE

After adjustment for age and education (in the MoVIES cohort), and for age and literacy (in the Ballabgarh cohort), the OR (with 95% CI) for probable or possible AD with CDR stage of at least 1.0, in APOE*E4 carriers compared with noncarriers, was 2.26 (1.29-3.95) in the MoVIES cohort and 3.35 (1.20-9.39) in the Ballabgarh cohort aged 70 years or older. Odds ratios for all dementias and for CDR of at least 0.5 in both cohorts, including the entire Ballabgarh cohort (all age groups), are shown in Table 3. In the MoVIES data, controlling for selection (random vs volunteer) status by including it as an independent variable in the models barely changed the ORs or CIs.

Since the MoVIES subjects undergoing genotyping were all aged 70 years or older, we restricted cross-national comparisons across Indian and US cohorts to subjects in the category aged 70 years or older. The ORs reported for AD with CDR of at least 1.0 were not significantly different across cohorts (P = .25), to the extent that a difference could be detected with available power. Essentially, the APOE*E4 allele was associated with AD at similar strength in both cohorts.

#### APOE*E2 ALLELE

After adjustment for age and education (in the Monongahela Valley), and for age and literacy (in Ballabgarh), the OR for probable or possible AD with CDR stage of at least 1.0, given the presence of at least 1 APOE*E2 allele, was calculated for both cohorts. No significant association was found between APOE*E2 and AD as all CIs included 1, most likely because of lack of power. All models had adequate fit by Hosmer-Lemeshow goodness-of-fit test.28

### COMMENT

The frequencies of AD and the APOE*E4 allele were higher among those who underwent genotyping within our US sample than in our Indian sample. The APOE*E4 carrier status and the presence of probable or

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**Table 1. Frequencies of Dementia, AD, and APOE Alleles**

<table>
<thead>
<tr>
<th>Age, y</th>
<th>No. of Subjects</th>
<th>Overall Dementia, CDR Stage</th>
<th>Probable or Possible AD, CDR Stage</th>
<th>APOE Allele Frequencies</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0.5</td>
<td>≥1.0</td>
<td>0.5</td>
</tr>
<tr>
<td>Ballabgarh, India, cohort</td>
<td>55-69</td>
<td>3203</td>
<td>(0.09)</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>70-79</td>
<td>971</td>
<td>(2.0)</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>≥80</td>
<td>276</td>
<td>(2.7)</td>
<td>12</td>
</tr>
<tr>
<td>Total</td>
<td>4450</td>
<td>7</td>
<td>(0.2)</td>
<td>29</td>
</tr>
<tr>
<td>Monongahela Valley, Pa, cohort</td>
<td>70-79</td>
<td>541</td>
<td>19</td>
<td>(3.5)</td>
</tr>
<tr>
<td></td>
<td>≥80</td>
<td>345</td>
<td>18</td>
<td>(11.0)</td>
</tr>
<tr>
<td>Total</td>
<td>886</td>
<td>57</td>
<td>(6.4)</td>
<td>75</td>
</tr>
</tbody>
</table>

*AD indicates Alzheimer disease; APOE, gene for apolipoprotein E; and CDR, Clinical Dementia Rating. Cohorts are described in the “Subjects and Methods” section.

**Table 2. Frequencies of APOE Genotype by Diagnosis of Dementia and Probable or Possible AD**

<table>
<thead>
<tr>
<th>APOE Genotype</th>
<th>Monongahela Valley, Pa, Cohort</th>
<th>Ballabgarh, India, Cohort</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total Sample</td>
<td>All Dementias</td>
</tr>
<tr>
<td><em>E2</em>E2</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td><em>E2</em>E3</td>
<td>103</td>
<td>14</td>
</tr>
<tr>
<td><em>E3</em>E3</td>
<td>595</td>
<td>84</td>
</tr>
<tr>
<td><em>E3</em>E4</td>
<td>159</td>
<td>31</td>
</tr>
<tr>
<td><em>E2</em>E4</td>
<td>16</td>
<td>1</td>
</tr>
<tr>
<td><em>E4</em>E4</td>
<td>11</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>886</td>
<td>132</td>
</tr>
</tbody>
</table>

*Abbreviations are given in the footnote to Table 1. Cohorts are described in the “Subjects and Methods” section of the text.
possible AD were positively associated in both cohorts. In each cohort, the elevated risk was about 2- to 3-fold, ie, the ORs were lower than those reported from the highly selected populations of Alzheimer's Disease Centers and similar referral centers.21,29,30 The ORs in our cohorts were within the range reported from other community-based studies of white populations in East Boston31 and Rotterdam,32 which included mild dementia, although lower than those from Framingham,33 which included only moderate to severe dementias. None of the previous reports included diagnostic information on AD or other diseases. Although ours is the largest Indian sample from which APOE allele frequencies have been reported, it is not representative of the population of India as a whole.

On the basis of a multicenter meta-analysis, Farrer et al7 concluded that the APOE*E4 allele “represents a major risk factor for AD in all ethnic groups studied, across all ages between 40 and 90 years, and in both men and women.” Studies included in the meta-analysis were of ethnic groups described as Caucasian, African American, Hispanic, and Japanese. Our US study population was white, a term often used interchangeably with Caucasian. However, our nonwhite Asian Indian population would also have to be classified as Caucasian, as natives of India and western Asia are described as forming “a Caucasoid cluster when compared with Asians from further north and east.”41 Previous genetic studies have suggested that linguistic (ie, ethnic) differences account for much of the genetic diversity of present-day Indian populations42,43; the ethnocultural diversity of present-day Indian populations is politically divided into linguistic states. Our study was conducted in Haryana, a northern Indian state whose inhabitants speak an Indo-European language (the Haryanvi dialect of Hindi). A study of Indians in 2 adjacent states, where the Punjabi and Hindi languages are spoken, demonstrated Caucasoid genetic features in addition to at least 1 marker suggesting ancient East Asian lineage.32 Thus, there is likely to be genetic admixture even in our Indian study population, which is probably more ethnically homogeneous than our Monongahela Valley study population of mixed European ancestry. Cross-ethnic comparisons can reveal important environmental differences (including, eg, cultural, geographic, socioeconomic, or dietary factors) that may be relevant to disease and that may interact with genetic risk. However, the genetic basis of conventional racial and ethnic distinctions per se is far from clear.42 Given the recent proliferation in reports of genetic studies from unique national, geographic, linguistic, racial, and ethnic groups, caution is warranted in comparing genetic data between studies that use different or overlapping ethnic classifications.

Our study had some limitations. The current report excluded the 17 African American subjects in our US genotyped cohort because of their small numbers. In both cohorts, the category of all dementias was

| Table 3. Associations Among APOE*E4, All Dementias, and AD* |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|
|                              | Cohort, Age     | No. of Subjects | No. of Cases   | Odds Ratio (95% CI)†  | P    |
| Ballabgarh, India, aged ≥55 y  | Probable or possible AD‡ | 4442 | 28 | 2.63 (1.12-6.15) | .03 |
|                                | All dementias   | 4450 | 36 | 2.44 (1.15-5.18) | .02 |
| Ballabgarh, aged ≥70 y         | Probable or possible AD‡ | 1244 | 22 | 3.15 (1.23-8.10) | .02 |
|                                | All dementias   | 1247 | 25 | 3.15 (1.30-7.61) | .01 |
| Monongahela Valley, Pa, aged ≥70 y | Probable or possible AD‡ | 869  | 115 | 1.70 (1.05-2.76) | .03 |
|                                | All dementias   | 886  | 132 | 1.48 (0.93-2.38) | .10 |

*CI indicates confidence interval. Other abbreviations are given in the "Subjects and Methods" section.
†Multiple logistic regression models were adjusted for age and educational level in the Monongahela Valley data and for age and literacy in the Ballabgarh data.
‡By criteria from the National Institute of Neurological and Communicative Disorders and Stroke–Alzheimer's Disease and Related Disorders Association for clinical diagnosis; other dementias were excluded from this model (hence smaller sample).
largely composed of probable or possible AD; we had too few cases of non-AD dementias to allow separate analyses of their relationship with APOE. As with most community-based studies, both cohorts included too few *E4/*E4 homozygotes to allow the exploration of dose-dependent risk of AD conferred by APOE*E4. Although the US cohort at its inception was representative of its base population, some natural attrition had occurred during the 6 years of follow-up before genotyping was performed. Although our clinical and genetic measures were comparable across the Indian and US cohorts, the two cohorts are very different from each other in a variety of cultural and environmental aspects that cannot easily be compared. Despite these differences, the cohorts revealed similar strength of association between APOE*E4 and AD; thus, the difference in disease occurrence between both study populations cannot be explained by differential risk, or modification of risk, with respect to the APOE polymorphism. As alternative explanations, differences in additional genetic risk and protective factors, survival effects, or environmental factors are promising directions for future research.

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Reprints: Mary Ganguli, MD, MPH, Western Psychiatric Institute and Clinic, 3811 O’Hara St, Pittsburgh, PA 15213-2593 (email: gangulim@msx.upmc.edu).

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