Prospective Study of Apolipoprotein E Genotype and Functional Outcome Following Ischemic Stroke

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**Background:** The apolipoprotein E (APOE) e4 allele is a marker of adverse outcome following head injury and intracerebral hemorrhage. Transgenic animal data in a focal cerebral ischemia model suggest that the e4 allele increases infarct size and functional impairment.

**Objective:** To determine if APOE genotype is associated with functional recovery from ischemic stroke.

**Design:** Prospective study.

**Setting:** Stroke service at a university teaching hospital.

**Patients:** Patients with clinical and neuroimaging findings (computed tomography or magnetic resonance imaging) compatible with an acute ischemic stroke.

**Main Outcome:** Functional outcome by Barthel index (BI) and modified Rankin scale (mRS) was compared for e3/e3 patients vs e4 carriers and vs e2 carriers at 1 and 3 months. Univariate predictors of 3-month outcome were examined in a multivariate analysis.

**Results:** One hundred eighty-nine patients were enrolled: 100 women, 89 men (mean±SD age, 69.4±11.0 years). There were 25 e2 alleles (frequency, 0.07), 292 e3 alleles (0.77), and 61 e4 alleles (0.16). Baseline National Institutes of Health Stroke Scale scores and Oxfordshire Community Stroke Project classifications were similar in all groups (e3/e3, e4, and e2 carriers). One-month (BI, P=.64; mRS, P=.59) and 3-month (BI, P=.87; mRS, P=.73) outcomes were not associated with possession of either e4 or the e2 allele. Baseline National Institutes of Health Stroke Scale scores (P<.001) and age (P=.002) were significant predictors of 3-month BI and mRS outcomes in multivariate analyses.

**Conclusions:** Although there is a robust influence of APOE polymorphism on functional recovery after some types of brain injury in humans, it does not exert a major influence on injury severity or functional recovery following ischemic stroke.

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PATIENTS AND METHODS

PATIENT SELECTION

Consecutive patients were recruited prospectively from an acute stroke unit between January 1997 and May 1998. Ischemic stroke was diagnosed when patients presented with an acute onset of a focal neurological deficit with no evidence of intracerebral hemorrhage or alternative pathologic characteristics on brain imaging. Prior to enrollment, all patients or a relative gave informed consent for the study. Clinical baseline characteristics including National Institutes of Health Stroke Scale (NIHSS) scores15 and Oxfordshire Community Stroke Project (OCSP) classifications16 were recorded at the time of admission. The study was approved by the hospital ethics committee.

APOE GENOTYPING

Apolipoprotein E genotypes were determined from whole blood samples by physicians blind to stroke classification and outcome. Leukocyte DNA was extracted and amplified by a polymerase chain reaction.17 The product was digested using the restriction enzyme HhaI, separated on a 10% polyacrylamide gel, stained with ethidium bromide, and visualized by UV light.

FOLLOW-UP

Participating patients were observed prospectively. Functional outcome was measured at 1 month and 3 months using the Barthel Index (BI)18 and modified Rankin scale (mRS).19,20 blind to APOE genotype.

STATISTICAL ANALYSIS

Differences in stroke severity using OCSP classifications and the NIHSS scores among different APOE genotype categories (ε3/ε3, ε2 carrying, and ε4 carrying patients) were assessed with a χ² test and Mann-Whitney test, respectively. Outcome categories of the BI and mRS were defined as in the National Institute of Neurological Disorders recombinant tissue plasminogen activator trial21: ie, good (mRS, 0-1; BI 95-100); moderate (mRS, 2-3; BI, 55-90); poor (mRS, 4-5; BI, 0-50); and dead (mRS, 6). Outcome was compared between ε4 carriers and ε3/ε3 patients as well as between ε2 carriers and ε3/ε3 patients using the χ² test. The groups were also examined as ε4 and non-ε4 carriers with outcome dichotomized as good (mRS, 0, 1, or 2; BI >55) vs all other functional categories. Differences in the distribution of potential prognostic variables (OCSP classifications and NIHSS scores; history of hypertension, stroke or transient ischemic attack, diabetes mellitus, atrial fibrillation, ischemic heart disease, peripheral vascular disease; current cigarette smoker; current infection or hyperlipidemia; use of aspirin, calcium antagonists, angiotensin-converting enzyme inhibitors; age; and possession of the ε2 or ε4 APOE alleles) were examined in a linear regression analysis using 3-month BI and mRS scores as categorical values. Variables with P <.05 on univariate analysis were then considered in a multivariate analysis by multiple linear regression.

RESULTS

One hundred eighty-nine patients were enrolled and APOE genotyped. All had follow-up assessments. There were 89 men and 100 women (mean ± SD age, 69.4 ± 11.0 years; range, 28-93 years). The baseline characteristics, including the OCSP classifications are given in Table 1. The median NIHSS score on admission was 6 (interquartile range, 3-10).

The APOE alleles were in Hardy-Weinberg equilibrium. No patient had the ε2/ε2 genotype, as indicated on the tabulation below.

<table>
<thead>
<tr>
<th>APOE Genotype</th>
<th>No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ε2/ε2</td>
<td>0</td>
</tr>
<tr>
<td>ε2/ε3</td>
<td>17 (9)</td>
</tr>
<tr>
<td>ε2/ε4</td>
<td>8 (4.2)</td>
</tr>
<tr>
<td>ε3/ε3</td>
<td>114 (60)</td>
</tr>
<tr>
<td>ε3/ε4</td>
<td>47 (25)</td>
</tr>
<tr>
<td>ε4/ε4</td>
<td>3 (1.6)</td>
</tr>
</tbody>
</table>

Fifty-eight patients (31%) carried 1 or more ε4 alleles and 25 (13%) had an ε2 allele. There was no significant difference in the age of ε3/ε3 patients (mean age, 69 years) and ε4 (mean age, 68 years; P = .56) or ε2 carrying patients (mean age, 73 years; P = .11) at the time of stroke. The APOE allele frequencies were 0.07 for ε2 (n = 25 alleles), 0.77 for ε3 (n = 292), and 0.16 for ε4 (n = 61), a similar frequency distribution to that found in previously studied Scottish populations.22

STROKE SEVERITY

There was no statistically significant difference in baseline stroke severity among the proportion of patients in different OCSP categories for ε3/ε3 vs ε2 or ε4 carriers (Table 2). Although patients possessing an ε4 allele had greater median NIHSS scores on admission to the hospital than ε3/ε3 carriers, the difference was also not statistically significant (P = .10).

OUTCOME

One-month outcome (Figure 1) was not statistically different in ε4 carriers and patients with the ε3/ε3 genotype for either BI (P = .64) or mRS (P = .59) scores. Similarly, there were no significant differences between ε2 carriers and ε3/ε3 patients (mRS, P = .19; BI, P = .87). The results were unchanged at 3 months (Figure 2). In addition, dichotomizing around end points that signify functional independence (mRS <3 and BI >55) yielded no statistically significant differences between ε4
analyses did not alter any of the results.

†Pearson $\chi^2$ test used to compare $e^3$ carriers compared with non-$e^3$ patients.

‡Mann-Whitney U test used to compare $e^2$ carriers compared with non-$e^2$ carriers.

*NIHSS indicates National Institutes of Health Stroke Scale; OCSP, Oxfordshire Community Stroke Project; TACI, total anterior circulation infarct; PACI, partial anterior circulation infarct; POCI, posterior circulation infarct; LACI, lacunar circulation infarct; CVA, cerebrovascular accident; TIA, transient ischemic attack; DVT, deep venous thrombosis; PE, pulmonary embolus; and ACE, angiotensin-converting enzyme.

Table 2. Stroke Severity and Apolipoprotein E Genotype*  

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>$e^3/e^3$ (n = 114)</th>
<th>$e^4$ (n = 58)</th>
<th>$e^2$ (n = 25)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median NIHSS score (interquartile range)†</td>
<td>5 (3-9)</td>
<td>7 (4-13)</td>
<td>5 (4-14)</td>
</tr>
<tr>
<td>OCSP classification, ‡</td>
<td>TACI 19 (17)</td>
<td>15 (26)</td>
<td>4 (16)</td>
</tr>
<tr>
<td>PACI 32 (28)</td>
<td>21 (36)</td>
<td>7 (28)</td>
<td></td>
</tr>
<tr>
<td>POCI 15 (13)</td>
<td>5 (9)</td>
<td>3 (12)</td>
<td></td>
</tr>
<tr>
<td>LACI 48 (42)</td>
<td>17 (29)</td>
<td>11 (44)</td>
<td></td>
</tr>
</tbody>
</table>

*NIHSS indicates National Institutes of Health Stroke Scale; OCSP, Oxfordshire Community Stroke Project classification; TACI, total anterior circulation infarct; PACI, partial anterior circulation infarct; POCI, posterior circulation infarct; LACI, lacunar circulation infarct.  
†Mann-Whitney U test used to compare $e^3$ patients with $e^3$ patients possessing $e^4$  
‡Pearson $\chi^2$ test used to compare $e^3$ patients with $e^3$ patients possessing $e^4$.
that the \(\epsilon 4\) allele does not exert a major adverse influence on baseline severity, functional outcome, or survival following ischemic stroke. These contrasting findings from different types of brain injury suggest that the association of the \(\epsilon 4\) allele with outcome may be insult specific.

Previous case-control and cohort studies have examined APOE genotype as a possible genetic predisposition to ischemic stroke.\(^{23-31}\) Some studies have implicated the \(\epsilon 2\) allele,\(^ {24,25}\) some the \(\epsilon 4\) allele,\(^ {25-27}\) and others have reported neutral findings.\(^ {28-31}\) A recent meta-analysis of case-control studies found a small significant overrepresentation of the \(\epsilon 4\) allele in ischemic stroke patients compared with age- and sex-matched controls.\(^ {32}\) The \(\epsilon 4\) allele is also overrepresented in coronary heart disease\(^ {33}\) and is thought to be more atherogenic than other APOE alleles.\(^ {31}\) Individuals with the \(\epsilon 3/\epsilon 4\) and \(\epsilon 4/\epsilon 4\) genotypes seem to carry excess risk compared with control subjects in both coronary heart disease\(^ {33}\) and ischemic stroke.\(^ {32}\) Although the results of the current study demonstrated fewer lacunar infarcts in \(\epsilon 4\) carriers (29% vs 42%) and higher median NIHSS scores compared with \(\epsilon 3/\epsilon 3\) patients (7 vs 5), neither of these was statistically significant, supporting the findings of a larger study that demonstrated no difference in APOE allele frequencies between patients with lacunar and cortical ischemic events.\(^ {10}\)

Properties of apoE that are potentially relevant to brain injury (neurotrophic,\(^ {35}\) antioxidant,\(^ {36}\) immunomodulatory,\(^ {37}\) and intracellular calcium effects\(^ {38}\)) have been identified under in vitro conditions. Although some of these are isoform specific (neurotrophic,\(^ {35}\) antioxidant,\(^ {36}\) and intracellular calcium changes\(^ {38}\)), the relevance in vivo of differing mechanisms has not been clearly defined. Consequently, in head injury and intracerebral hemorrhage (conditions for which possession of the \(\epsilon 4\) allele seems to almost double the risk of a poor prognosis\(^ {10,19}\)), it is not known whether \(\epsilon 4\) carriers incur more severe injury or have defective repair mechanisms compared with non-\(\epsilon 4\) carriers. In the present study, baseline NIHSS scores and OCSP categories were used to assess stroke severity. Similar findings in each of the major APOE genotype groupings (\(\epsilon 3/\epsilon 3\), \(\epsilon 4\), and \(\epsilon 4\) carriers) suggest that ischemic insult severity in stroke is not associated with APOE genotype.

The outcome findings of our study question how reliably one can extrapolate data from animal models of stroke to humans.\(^ {39}\) Brain apoE changes have been well described in different animal models of brain injury,\(^ {1,10}\) including cerebral ischemia.\(^ {41}\) The results have suggested that injury either causes neuronal expression of apoE or increases neuronal uptake of apoE.\(^ {41}\) This has led to the description of apoE as an “injury factor.”\(^ {42}\) In one focal ischemic model, infarct volume and hemiparesis severity were significantly worse in transgenic \(\epsilon 4/\epsilon 4\) mice compared with \(\epsilon 3/\epsilon 3\) mice.\(^ {44}\) It remains unclear if APOE genotype influences the size of human infarct volume in different types of ischemic stroke. Tomimoto et al\(^ {10}\) have demonstrated that apoE-immunoreactive axons in humans are accompanied by apoE-positive macrophages in the periphery of infarcts, although APOE genotypes were not examined in this small study. However, a recent semiquantitative assessment of neuronal damage following global ischemia in humans failed to demonstrate an APOE genotype influence.\(^ {44}\)

The follow-up functional assessments in our study provided a means, albeit crude, of measuring repair. The neutral results suggest that the different apoE isoforms do not reflect major differences in repair mechanisms following cerebral ischemia in humans. A trend toward better functional outcome in \(\epsilon 2\) carriers vs either the \(\epsilon 3/\epsilon 3\) or \(\epsilon 4+\) groups was present, but must be interpreted with caution since only 13% of the population (n=25 patients) possessed an \(\epsilon 2\) allele. Furthermore, survival and 3-month placement following ischemic stroke in a cohort of 640 patients\(^ {10}\) were not associated with the APOE \(\epsilon 2\) allele.

Although ischemic stroke is a heterogeneous condition, the number of patients in our study is much greater than in those studies of head injury\(^ {5,10}\) or intracerebral hemorrhage\(^ {5,10}\) (equally heterogeneous conditions) that demonstrated an influence of APOE \(\epsilon 4\) on outcome. This supports the conclusion that APOE genotype does not contribute to major clinically significant differences in either injury severity or functional outcome in ischemic stroke. Our results indicate that associations between APOE genotype and outcome in humans may be insult specific.

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