Prevalence of Apolipoprotein E Alleles in Healthy Subjects and Survivors of Ischemic Stroke
An Italian Case-Control Study

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Background and Purpose—The ε4 allele of the apolipoprotein E (apoE) has been related to the occurrence of myocardial infarction, but its association with ischemic stroke is controversial. We have evaluated the relation between apoE alleles and the occurrence of cerebrovascular ischemia.

Methods—The apoE ε genotypes of 100 patients with a documented history of ischemic stroke without clinically apparent dementia (stroke+) and 108 subjects without such history (stroke−) were determined. The relative frequency of the apoE alleles and genotypes was estimated in 398 healthy subjects aged <40 years from the same ethnic background.

Results—The frequency of the apoE ε4 allele in stroke+ (0.18 [95% CI, 0.12 to 0.25]) was higher than in stroke− (0.07 [95% CI, 0.03 to 0.12]; P<.001) or in healthy subjects (0.09 [95% CI, 0.07 to 0.12]; P<.001). Carriers of the ε4 allele differed between stroke+ (0.30 [95% CI, 0.19 to 0.42]) and stroke− (0.12 [95% CI, 0.5 to 0.22]; P=.004) or healthy subjects (0.16 [95% CI; 0.12 to 0.22]; P=.015). Accordingly, ε3/ε3 homozygotes were less frequent in stroke+ (0.59 [95% CI, 0.45 to 0.71]) than in stroke− (0.72 [95% CI, 0.59 to 0.82]; P=.063) or in healthy subjects (0.73 [95% CI, 0.67 to 0.78]; P=.01). In a multiple logistic regression analysis, age (P<.03), positive family history (P<.04) and apoE (P<.002) independently contributed to a stroke history, with ε4 carriers exhibiting a higher estimated risk (odds ratio, 5.05).

Conclusions—Our data show an association between apoE gene and a personal history of ischemic stroke and support the possibility that the apoE gene is a susceptibility locus for the risk of cerebrovascular ischemic disease. (Stroke. 1998;29:399-403.)

Key Words: apolipoprotein E ■ risk factors ■ stroke ■ thrombosis

Stroke is a major complication of atherosclerotic cardiovascular disease and a leading cause of morbidity and mortality in Western countries. Individuals who smoke, have high blood pressure or high plasma levels of glucose, or are obese are all at risk for this event.1-5 However, such risk factors account for only about one third of the future ischemic episode.6-8 Apolipoprotein E (apoE) is a polymorphic glycoprotein that plays a critical role in cholesterol homeostasis and in the catabolism of triglyceride-rich lipoproteins.9,10 ApoE occurs in plasma in six common isoforms, encoded by three alleles: ε2, ε3, and ε4. ApoE ε3 is the predominant isoform, with the other two isoforms differing from apoE ε3 for amino acid substitutions at position 112 (ε4: Cys→Arg) or at position 158 (ε2: Arg→Cys).11,12 Several reports have found a relationship between apoE alleles and early development of atherosclerosis,13,14 ischemic coronary heart disease,15-17 or cerebrovascular disease.18,19 In a recent meta-analysis20 the association between apoE ε4 allele and a high risk of cardiovascular ischemic events in men and in women was confirmed and extended. Likewise, the association between the ε4 allele and late-onset Alzheimer’s disease is now well established.21,22 In contrast, the association between ε420 and ε220 alleles and ischemic stroke is still debated.23-25

In our setting of survivors of ischemic stroke devoid of any clinically detectable symptom of dementia, we have investigated the prevalence of apoE alleles and compared the results with those found in a large group of healthy subjects from the same geographic area.

Subjects and Methods

Subjects
After approval of the Ethics Committee, our studies were carried out according to the Principles of the Declaration of Helsinki; informed consent was obtained from all subjects. From February to December 1992, 210 subjects (108 men and 102 women; mean age, 63.6 years [range 31 to 86 years]) were enlisted for the study. They were chosen from among subjects who had been attending the metabolic ward of...
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the outpatient Clinic of our Institution. From 8 to 12 months before being enlisted, 101 of them (51 males and 50 females) had survived an ischemic stroke. Demographic characteristics of the subjects, the manner in which they were enlisted (inclusion/exclusion criteria), and similarities and differences among cases (stroke+) and controls (stroke−) have been reported elsewhere. This population was free of mental impairment as assessed by the Mini-Mental State Exam. None of the 210 subjects had clinical evidence of cancer or acute or chronic inflammatory disease. All had been repeatedly instructed to stop smoking and drinking alcohol and to control food intake, and all were highly motivated to follow the advice. All had been on an isocaloric Mediterranean-style diet for at least 6 months. A complete clinical summary with emphasis on personal and family history for angina pectoris, myocardial infarction, ischemic stroke, peripheral arterial disease, and vascular risk factors was obtained from all subjects. Positive family history was defined as the occurrence of stroke or myocardial infarction before the age of 55 in male and 60 in female parents and siblings. The 109 stroke− subjects were comparable to stroke− individuals with respect to sex, height, occupation, social class, and risk factors for coronary artery disease. In particular, no difference between stroke+ and stroke− individuals was found with respect to mean plasma concentrations of total, HDL, and LDL cholesterol; triglycerides; and Lp(a). Neither were differences found in the number of subjects with documented history of ischemic stroke. Demographic characteristics of the subjects, the economic status, and the occupation of the two groups could not be obtained.

The relative frequency of the apoE alleles and genotypes was estimated in 398 healthy subjects (168 men and 230 women) aged <40 years and from the same ethnic background. In particular, they were free of personal history of cardiovascular and neurovascular disease.

Materials
dNTP, KCl, MgCl2, gelatin, agarose, and mineral oil were from Perkin-Elmer Cetus. Proteinase K was obtained from USB Corp.; l hypophosphat (d=1.077), from Nyegaard; and HEPES, Tris-HCl, EDTA, ethidium bromide, and SDS from Sigma Chemical Co. We collected 18 mL of blood from each subject at 9 to 9:30 AM (after 12 to 15 hours of overnight fasting) without venous stasis from the antecubital vein via a 19-gauge scalp vein needle. The blood was placed in a sterile tube containing 2 mL of sterile 3.8% trisodium citrate and processed immediately. Concentrations of total cholesterol, HDL cholesterol, triglycerides, and plasma glucose were detected enzymatically20 with use of commercially available reagents (Roche). The Friedewald equation (total cholesterol = HDL cholesterol + triglycerides×5) was used to calculate concentrations of LDL cholesterol.

Isolation of DNA and Genotype Analysis
Peripheral blood leukocytes were incubated overnight at 37° C in a digestion buffer (100 mmol/L NaCl, 10 mmol/L Tris-HCl, 11.963; P<.001). Accordingly, the frequency of the e3 allele was lower in stroke+ (0.76 [95% CI; 0.68 to 0.83] versus 0.85 [95% CI; 0.78 to 0.90] in stroke−; χ2 = 5.065; P = .024). A similar figure was observed when stroke+ data were compared with those observed in a group of healthy subjects <40 years of age (Table 1). The distribution of the genotype frequencies differed significantly between stroke+ and the other two settings and slightly between the two control groups (Table 1). The frequency of the e3/e4 genotype was different in stroke+ and stroke− individuals. Likewise, the number of e3/e3 subjects varied significantly between stroke+ individuals and healthy subjects. Finally, the e4 carriers were more common in the stroke+ setting than in stroke− or in healthy subjects (χ2 = 8.30, P = .004, and χ2 = 5.93, P = .015, respectively). The genotype frequencies were not different from those predicted from the Hardy-Weinberg equilibrium in stroke+ (χ2 = 3.559; P = .724), in stroke− (χ2 = 1.432; P = 1.0), and in healthy subjects (χ2 = 4.44; P = 0.294). In addition to a positive family history for cardiovascular events and age >70 years, a carrier state of the e4 allele was more frequent in stroke+ than in stroke− individuals (Table 2). Accordingly, e3/e3 homozygotes were less common in stroke+ than in stroke− individuals. The ORs of having a history of stroke were 3.13 (95% CI; 1.52 to 6.44) and 0.55 (95% CI; 0.31 to 0.99) for the e4 allele and e3/e3 genotype, respectively. No differences were found between stroke+ and stroke− individuals in the frequency of hypertension (56% versus 55%), diabetes mellitus (27% versus 33%), and LDL cholesterol >3.4 mmol/L (24% versus 25%).

Selected Abbreviations and Acronyms
apoE = apolipoprotein E
CI = confidence interval
OR = odds ratio
stroke− = study patients without documented history of ischemic stroke
stroke+ = study patients with documented history of ischemic stroke

Statistical Analysis
All the analyses were performed according to the SPSS/PC V2.0 statistical package, following the recommended procedures. The Kolmogorov-Smirnov test, a nonparametric method, was used to compare the distributions of the variables in stroke+ and stroke− subjects. Pearson’s χ2 statistic was used to evaluate the independent nature of the clinical condition with respect to categorical variables. ORs and 95% CIs were calculated. Appropriate models were set up to evaluate in a logistic analysis the independent contribution of each variable to the ischemic event. An enter method was used to set up the system; the log likelihood and Wald χ2 statistics are presented. For all the tests, significance was established at P<.05.

Results
In the stroke+ group, the frequency observed for the allele e4 was significantly higher than that observed in stroke− individuals (0.18 [95% CI; 0.12 to 0.25] versus 0.07 [95% CI; 0.03 to 0.12]; χ2 = 11.963; P<.001). Accordingly, the frequency of the e3 allele was lower in stroke+ (0.76 [95% CI; 0.68 to 0.83] versus 0.85 [95% CI; 0.78 to 0.90] in stroke−; χ2 = 5.065; P = .024). A similar figure was observed when stroke+ data were compared with those observed in a group of healthy subjects <40 years of age (Table 1). The distribution of the genotype frequencies differed significantly between stroke+ and the other two settings and slightly between the two control groups (Table 1). The frequency of the e3/e4 genotype was different in stroke+ and stroke− individuals. Likewise, the number of e3/e3 subjects varied significantly between stroke+ individuals and healthy subjects. Finally, the e4 carriers were more common in the stroke+ setting than in stroke− or in healthy subjects (χ2 = 8.30, P = .004, and χ2 = 5.93, P = .015, respectively). The genotype frequencies were not different from those predicted from the Hardy-Weinberg equilibrium in stroke+ (χ2 = 3.559; P = .724), in stroke− (χ2 = 1.432; P = 1.0), and in healthy subjects (χ2 = 4.44; P = 0.294). In addition to a positive family history for cardiovascular events and age >70 years, a carrier state of the e4 allele was more frequent in stroke+ than in stroke− individuals (Table 2). Accordingly, e3/e3 homozygotes were less common in stroke+ than in stroke− individuals. The ORs of having a history of stroke were 3.13 (95% CI; 1.52 to 6.44) and 0.55 (95% CI; 0.31 to 0.99) for the e4 allele and e3/e3 genotype, respectively. No differences were found between stroke+ and stroke− individuals in the frequency of hypertension (56% versus 55%), diabetes mellitus (27% versus 33%), and LDL cholesterol >3.4 mmol/L (24% versus 25%).
As many as 48 patients had an atherothrombotic infarction, 19 an embolic stroke, 10 a lacunar infarction, and 10 an ischemic episode involving a boundary region. The remaining 13 experienced an ischemic stroke of undetermined type. No association was found between apoE ε alleles and type of ischemic stroke. No differences with respect to the apoE ε alleles were found with respect to sex, age above or below 70 years, hypertension, diabetes mellitus, family history of ischemic events, t-PA >10 ng/dL, or PAI-1 levels.

The independent nature of the contribution of the ε4 allele to a stroke history was assessed in a multiple logistic regression model in which, in addition to the apoE polymorphism, a series of relevant covariates were included (Table 3). The analysis showed a significant excess (OR, 5.05; 95% CI, 1.82 to 14.01) of a personal history of cerebrovascular disease in apoE ε4 carriers compared with ε4 noncarriers, confirming the strength of the association observed in the univariate analysis (OR, 3.13).

**Discussion**

In a setting of patients with a personal history of ischemic stroke (stroke+) who differed from controls without such a history (stroke−) for age and familial history of cardiovascular and cerebrovascular diseases, we found a strong and independent relation between the personal history and the ε4 carrier status. Our calculated ORs reflect only the association between the apoE ε4 genotype and a personal history of cerebrovascular ischemia. However, apoE is a major lipoprotein involved in cholesterol metabolism. The association between atherosclerosis and ischemic risk confers a biological plausibility to our findings.

These data support some previous reports in this area and dispute others.19–23 Such inconsistencies may be due to the association of apoE ε4 allele with late-onset Alzheimer’s disease.21 In this respect, we enlisted subjects without clinically apparent mental impairment. However, the relation between apoE ε4 and the risk of dementia with stroke was also found in a setting with vascular dementia only, in which a

### TABLE 1. ApoE Allele and Genotypes According to the History of Ischemic Stroke

<table>
<thead>
<tr>
<th>Allele</th>
<th>Healthy Subjects (n=796)</th>
<th>Stroke− (n=216)</th>
<th>Stroke+ (n=200)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ε2</td>
<td>46 (0.06 [0.04–0.08])</td>
<td>18 (0.08 [0.04–0.14])</td>
<td>12 (0.06 [0.03–0.10])</td>
</tr>
<tr>
<td>ε3</td>
<td>677 (0.85 [0.82–0.88])*</td>
<td>104 (0.85 [0.78–0.90])†</td>
<td>152 (0.76 [0.68–0.83])</td>
</tr>
<tr>
<td>ε4</td>
<td>73 (0.09 [0.07–0.12])‡</td>
<td>14 (0.07 [0.03–0.12])§</td>
<td>36 (0.18 [0.12–0.25])</td>
</tr>
</tbody>
</table>

Genotype

<table>
<thead>
<tr>
<th>Allele</th>
<th>Healthy Subjects (n=398)</th>
<th>Stroke− (n=108)</th>
<th>Stroke+ (n=100)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ε2/ε2</td>
<td>4 (0.01 [0.00–0.03])</td>
<td>1 (0.01 [0.00–0.06])</td>
<td>1 (0.01 [0.00–0.07])</td>
</tr>
<tr>
<td>ε2/ε3</td>
<td>31 (0.08 [0.05–0.12])</td>
<td>16 (0.15 [0.07–0.15])</td>
<td>10 (0.10 [0.04–0.20])</td>
</tr>
<tr>
<td>ε2/ε4</td>
<td>7 (0.02 [0.01–0.04])</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>ε3/ε3</td>
<td>290 (0.73 [0.67–0.78]) §§</td>
<td>78 (0.72 [0.59–0.82])¶</td>
<td>59 (0.59 [0.45–0.71])</td>
</tr>
<tr>
<td>ε3/ε4</td>
<td>66 (0.16 [0.12–0.22])</td>
<td>12 (0.11 [0.04–0.12])#</td>
<td>24 (0.24 [0.14–0.36])</td>
</tr>
<tr>
<td>ε4/ε4</td>
<td>0</td>
<td>1 (0.01 [0.00–0.06])</td>
<td>6 (0.06 [0.01–0.14])</td>
</tr>
</tbody>
</table>

Values are allele and genotype numbers observed (frequencies [95% CI]).

Healthy subjects vs stroke+ group: *P=.003; †P<.001; ‡P=.01; alleles χ²=12.972, P=.002; genotypes χ²=30.738, P<.001.

Healthy subjects vs stroke− group: genotypes χ²=17.759, P=.040.

Stroke− vs stroke+ groups: *P=.024; §P<.001; ¶P=.063; #P=.023; alleles χ²=13.332, P=.001; genotypes χ²=11.300, P=.023.

### TABLE 2. Study Subject Characteristics

<table>
<thead>
<tr>
<th>Variable</th>
<th>Stroke+ (n=100)</th>
<th>Stroke− (n=108)</th>
<th>OR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Men</td>
<td>51</td>
<td>57</td>
<td>0.93</td>
<td>0.54–1.60</td>
</tr>
<tr>
<td>Hypertension</td>
<td>56</td>
<td>59</td>
<td>1.05</td>
<td>0.61–1.82</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>27</td>
<td>36</td>
<td>0.74</td>
<td>0.41–1.34</td>
</tr>
<tr>
<td>LDL cholesterol &gt; 3.4 mmol/L</td>
<td>24</td>
<td>27</td>
<td>0.95</td>
<td>0.50–1.78</td>
</tr>
<tr>
<td>Positive family history</td>
<td>40</td>
<td>27</td>
<td>1.99</td>
<td>1.10–3.59</td>
</tr>
<tr>
<td>Age &gt; 70 y</td>
<td>52</td>
<td>35</td>
<td>2.26</td>
<td>1.29–3.97</td>
</tr>
<tr>
<td>ApoE ε4 allele</td>
<td>30</td>
<td>13</td>
<td>3.13</td>
<td>1.52–6.44</td>
</tr>
</tbody>
</table>

### TABLE 3. Factors Associated With a History of Ischemic Stroke

<table>
<thead>
<tr>
<th>Variable</th>
<th>B</th>
<th>SE</th>
<th>Wald χ²</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive family history</td>
<td>1.0881</td>
<td>0.5060</td>
<td>4.6243</td>
<td>0.0315</td>
</tr>
<tr>
<td>ApoE ε4 allele</td>
<td>1.6198</td>
<td>0.5205</td>
<td>9.6845</td>
<td>0.0019</td>
</tr>
<tr>
<td>Age, y</td>
<td>0.0515</td>
<td>0.0229</td>
<td>5.0781</td>
<td>0.0242</td>
</tr>
</tbody>
</table>

By logistic regression analysis. B indicates estimated coefficient; SE, standard error.

−2 log likelihood χ²=274.466; model χ²=148.619 (df=15, P<.0001).
portion of patients were diagnosed if the onset of dementia occurred within 3 months after stroke. \(^{20}\) Mean age of our stroke \(^+\) individuals (66.2 years) was slightly higher than that of our stroke \(\sim\) individuals (61.2 years) but lower than that of a French (72.3 years), \(^{10}\) a Spanish (71.2 years), \(^{23}\) and two Scandinavian series (68.9 and >80 years, respectively), \(^{21,22}\) and close to that (64.4 years) of a series \(^{15}\) in which a relation between apoE \(\epsilon 4\) and ischemic stroke was suggested. Age distribution may be an important confounding factor; risk factor profiles of ischemic stroke vary in various age classes. \(^{31}\) Differences in the control subject selection among studies may account, at least in part, for some of these discrepancies, particularly in view of the low frequency of the \(\epsilon 4\) allele. \(^{32}\) We selected control subjects (stroke \(\sim\) among inpatients without ischemic cardiovascular and cerebrovascular diseases. However, the differences between stroke \(\sim\) and stroke \(+\) individuals were comparable to those between stroke \(+\) and healthy subjects aged <40 years. In addition, \(\epsilon 4\) allele frequencies comparable to ours (<0.10) have been reported in other Italian series\(^{30,31}\) and also in elderly Italian controls. \(^{36}\) Population studies have demonstrated that the different ethnic and geographic distribution of apoE isoforms are associated with a different prevalence of dyslipidemia and coronary heart disease. \(^{36,37}\) Moreover, differences in stroke incidence among participating populations in the WHO MONICA project \(^{18}\) are quite similar to those observed in apoE gene frequencies showing a north-to-south gradient. In this respect, stroke incidence in Italian populations \(^{38–40}\) is consistently lower than those reported in other countries. \(^{38}\) In the present report, the distribution of the apoE genotypes slightly differs between stroke \(\sim\) and healthy subjects. However, allele frequencies were similar between the two groups. In our stroke \(\sim\) individuals, the frequency of the \(\epsilon 4\) allele was 0.07; ie, it did not differ from that (0.09) observed in a setting of 398 healthy individuals of both sexes aged <40 years \((\chi^2 = 1.24; P = .265)\). Nor were \(\epsilon 2\) and \(\epsilon 3\) alleles different in the two settings. Thus, the different distribution of the apoE \(\epsilon 3/\epsilon 4\) genotype between the two control settings may arise from variations by chance. Alternatively, the relative increase of \(\epsilon 2\) carriers and the decrease of \(\epsilon 4\) carriers among elderly stroke \(\sim\) subjects compared with the younger healthy group may suggest a role for the apoE polymorphism in the pathogenesis of ischemic stroke as age increases. Cross-sectional studies report a high association between the \(\epsilon 4\) allele and the severity of atherosclerosis. \(^{13,14,41}\) Thus, different survival rates among apoE genotypes have to be taken into account, as suggested by a reduction of the \(\epsilon 4\) allele among octogenarians and centenarians. \(^{43}\) In this respect, it has been suggested that apoE \(\epsilon 4\) affects stroke survival. \(^{20}\) Since we did not enlist cases at the time of the cerebrovascular event, the present study cannot address this issue. However, the cumulative probability of a person aged 45 years having an acute stroke increases by threefold (twofold in women) from 65 to 75 years of age and by eightfold (sixfold in women) from 75 to 85 years of age. \(^{41}\) Case-fatality rate depends on age structure. \(^{41}\) Thus, the relation between apoE \(\epsilon 4\) and ischemic stroke that we found is unlikely to be significantly affected by the selection of stroke survivors as cases.

Our data support the concept that apoE gene is a susceptibility locus; ie, it is neither necessary nor sufficient for the disease to occur but makes it more likely that one will become ill. The extent to which this polymorphism confers an additional cerebrovascular risk has to be addressed in prospective studies.

References


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Stroke. 1998;29:399-403
doi: 10.1161/01.STR.29.2.399

Stroke is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0039-2499. Online ISSN: 1524-4628

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