Synergistic Effect of Apolipoprotein E Polymorphisms and Cigarette Smoking on Risk of Ischemic Stroke in Young Adults

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Background and Purpose—The effect of apolipoprotein E (APOE) polymorphisms on stroke risk may be influenced by the coexistence of modifiable predisposing conditions. We explored the interactions of APOE genotypes and conventional risk factors in a case-control study of young adults with cerebral infarct.

Methods—We analyzed 124 consecutive patients (age, 34.7 ± 7.3 years) and 147 age- and sex-matched controls. APOE genotypes were determined by restriction fragment-length polymorphism analysis.

Results—The prevalence of the e4 allele and e34 genotype was slightly higher in cases than in controls (0.125 versus 0.071 and 0.242 versus 0.136, respectively). Carriers of the e34 genotype and e4 allele were associated with an increased risk of stroke on multivariate analysis compared with the e33 genotype and non-e4 carriers, respectively (odds ratio [OR], 2.29; 95% confidence interval [CI], 1.10 to 4.76; and OR, 2.27; 95% CI, 1.13 to 4.56). ORs for stroke were 2.99 (95% CI, 1.64 to 5.45), 2.69 (95% CI, 1.25 to 5.77), and 5.39 (95% CI, 1.59 to 18.30) for smokers with the e33 genotype, nonsmokers with the e34 genotype, and smokers with the e34 genotype, respectively, compared with nonsmokers with the e33 genotype. Similar results were obtained when e4 carriers and non-e4 carriers were compared in the same interaction model. No significant interaction between APOE and hypertension was found.

Conclusions—In young adults, the APOE e4 allele and cigarette smoking act synergistically, increasing an individual’s propensity to have a cerebral ischemic event. This finding may help in determining an individual’s predisposition to stroke and more targeted preventive interventions. (Stroke. 2004;35:438-442.)

Key Words: apolipoproteins ■ cigarette smoking ■ polymorphism ■ risk factors

Despite growing evidence suggesting that genetic factors play a key role in the pathogenesis of cerebrovascular disease, results from candidate gene studies have been conflicting to date.1 One reason is that ischemic stroke is probably the end phenotype of a complex interaction between environmental factors and an inherited background. The effect of single candidate polymorphisms on the risk of stroke may be weak when analyzed individually but may be more pronounced in the presence of modifiable risk factors.

The apolipoprotein E (APOE) gene is the most important modulator of plasma lipids and lipoprotein.2 Although the association between the e4 allele of this polymorphism and the risk of both ischemic heart disease and sporadic Alzheimer’s disease has been formally demonstrated, the role of the APOE genotype in ischemic stroke is controversial. This inconsistency may be due to methodological issues in study designs and to the heterogeneous nature of ischemic stroke. In addition, as data from twin studies and population studies have indicated, genetic influences may be more relevant in younger than in older individuals.1,3 Therefore, it is possible that the effect of the APOE polymorphism varies with age and may be influenced by an interaction with environmental determinants. Separate analyses for younger and older stroke patients have rarely been conducted in most of the previous association studies.4–6 Furthermore, no studies have evaluated whether the presence of conventional risk factors may modify the association between APOE genotypes and ischemic stroke.

In the present study, we investigated the potential relationship between cerebral ischemic stroke and the genetic polymorphisms of APOE in a case-control analysis of a series of young adults and tested the hypothesized interaction of conventional risk factors with APOE isoforms in stroke occurrence.

Subjects and Methods

Data were obtained in the setting of a single-center, hospital-based study designed for the evaluation of gene-environment interactions in the development of ischemic cerebrovascular disease. A detailed description of methods have been given elsewhere.7,8 Briefly, 125 unselected, consecutive, unrelated subjects with first-ever acute
ischemic stroke occurring before 45 years of age were entered into the group of cases. One hundred forty-nine subjects from the staff of our hospital with no known history of vascular disease who were matched to the cases by sex and age in 3-year bands served as controls. Both cases and controls were white and were from the same geographic area and social status.8

To investigate any potential association between APOE polymorphisms and specific subtypes of infarct, cases were divided into 2 subgroups on the basis of the presumed pathogenic mechanism according to a classification based on the Trial of Org 10172 in Acute Stroke Treatment (TOAST) criteria, accommodated and validated for the cause of stroke in the young.9 The first subgroup included subjects with infarction caused by large-vessel atherosclerotic vasculopathy or small-vessel disease (atherosclerotic); the second subgroup included subjects with infarction caused by other pathogenic mechanisms (nonatherosclerotic).

The study was designed and carried out in observance of the ethical principles established by the local Institutional Guidelines on Clinical Investigation. Written, informed consent was provided by all study participants.

Biochemical and Genetic Analyses
Venous blood sampling for biochemical determinations took place in the early morning (before 7 AM) after overnight fasting in all subjects. In patients, blood draws were performed 7 to 10 days after the acute event.

The APOE genotypes were determined according to the method of Hixson and Vernier.10 For technical reasons, APOE genotypes were not available in 3 subjects (1 case, 2 controls). Thus, data from 124 cases and 147 controls were entered into the final analysis.

Statistical Analysis
Differences in baseline characteristics between cases and controls and between the atherosclerotic and nonatherosclerotic subgroups were assessed by the χ² test. Bivariate mean differences (MDs), odds ratio (ORs), and 95% confidence intervals (CIs) were estimated for the acute event. ORs and CIs for ratio (ORs), and 95% confidence intervals (CIs) were estimated for

The 4×2 table approach11 was used to estimate the additive interaction between APOE genotypes and conventional risk factors. In the first model, nonsmokers with the e3/e3 genotype were the referent category and were compared with nonsmokers with the e3/e4 genotype, smokers with the e3/e3 genotype, and smokers with the e3/e4 genotype. The same model was also used to explore the interaction between e3 and e3 genotypes and hypertension. Finally, to examine the single-gene effect, separate models were generated including e4 carriers and non-e4 carriers, with nonsmokers–non-e4 carriers and nonhypertensive non-e4 carriers as referent categories. The modeling strategies included assessment of interaction without and with adjustment for covariates by logistic regression. The Rothman’s synergy (S) measure was also computed.12 The S index is the ratio of the observed effect with joint exposure divided by the effect predicted for joint exposure assuming additivity of the effects. No interaction corresponds to S = 1, whereas S > 1 (S < 1) can be interpreted as a measure of relative increase (decrease) in the effect among those exposed to both factors. Analyses were conducted with the SPSS (version 11.1) software package.

Results
Baseline characteristics of the study group are summarized in Table 1. Genotype frequencies did not differ significantly from those predicted by the Hardy-Weinberg equilibrium ([χ² (df) = 6.18(3); P = 0.102]. The proportion of subjects carrying the e3/e4 genotype among cases (24.2%) was slightly significantly higher than that among controls (13.6%). Similar results were obtained comparing the distribution of the e4 allele and the e4 carriers in the 2 groups (Table 2).

Both the e3/e4 genotype and e4 carrier status were associated with ~2-times-greater odds of ischemic stroke after multivariate model compared with the e3/e3 genotype and non-e4 carrier status (OR, 2.29; 95% CI, 1.10 to 4.76; and OR, 2.27; 95% CI, 1.13 to 4.56, respectively).

Smokers carrying the e3/e3 genotype had an increased risk of stroke compared with nonsmokers with the same genotype (OR, 2.99; 95% CI, 1.64 to 5.45). A similar effect was determined by the presence of the e3/e4 genotype alone (OR, 2.69; 95% CI, 1.25 to 5.77), whereas the combination of the e3/e4 genotype and current smoking was associated with 5.4-times-greater odds of ischemic stroke (OR, 5.39; 95% CI, 1.59 to 18.30). The combined effect of the e3/e4 genotype and smoking on stroke risk was ~20% greater than that predicted by assuming additivity of effects (S = 1.19). Adjustment for other covariates did not significantly change the results. Similar findings were obtained when e4 carriers were com-


TABLE 2. Characteristics of the Study Group According to APOE Genotype

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Stroke Cases (n=124)</th>
<th>Control Subjects (n=147)</th>
<th>Crude OR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>ε2</td>
<td>Cases (n=2)</td>
<td>Controls (n=13)</td>
<td>1.17</td>
<td>0.54–2.56</td>
</tr>
<tr>
<td>ε3</td>
<td>Cases (n=31)</td>
<td>Controls (n=125)</td>
<td>1.27</td>
<td>0.27–2.69</td>
</tr>
<tr>
<td>ε4</td>
<td>Cases (n=3)</td>
<td>Controls (n=22)</td>
<td>1.36</td>
<td>0.08–22.08</td>
</tr>
</tbody>
</table>

Allele

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Cases (n=113)</th>
<th>Controls (n=133)</th>
<th>Crude OR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>ε2 carrier</td>
<td>11 (8.9)</td>
<td>14 (9.5)</td>
<td>0.92</td>
<td>0.40–2.11</td>
</tr>
<tr>
<td>Non-ε2 carrier</td>
<td>113 (91.1)</td>
<td>133 (90.5)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>ε3 carrier</td>
<td>121 (97.6)</td>
<td>146 (99.3)</td>
<td>0.27</td>
<td>0.02–2.69</td>
</tr>
<tr>
<td>Non-ε3 carrier</td>
<td>3 (2.4)</td>
<td>1 (0.7)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>ε4 carrier</td>
<td>31 (25)</td>
<td>21 (14.3)</td>
<td>2.00</td>
<td>1.08–3.70</td>
</tr>
<tr>
<td>Non-ε4 carrier</td>
<td>93 (75)</td>
<td>126 (85.7)</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated with ε3 and ε4 carriers as reference categories.

In contrast, there was no evidence of genotype effect or single-gene effect among subjects with and without increased arterial blood pressure (Table 4).

Finally, although the frequency of both current smokers and the ε4 allele was slightly higher in the atherosclerotic subgroup of cases than in the nonatherosclerotic subgroup (18 of 31 [58%] versus 41 of 93 [44%], and 9 of 62 [14.5%] versus 22 of 186 [11.8%], respectively), the difference did not reach statistical significance. The same result was found when the frequency of current smokers carrying the ε4 allele was compared.

Discussion

The results of the present study suggest that (1) the APOE polymorphisms may be a moderate independent risk factor for ischemic stroke in young adults and (2) the APOE polymorphisms interact with smoking to increase the risk of ischemic stroke in young adults, prompting us to speculate a synergistic effect on the risk of stroke occurrence. In contrast, despite a trend toward a significant association, no relationship was observed between APOE and hypertension.

The potential link between APOE and ischemic cerebrovascular disease has been the subject of considerable debate, with some reports detecting negative associations4,13–15 and others demonstrating a modest increase of stroke risk by the ε4 allele.6,15,16 What is relevant to the results of the present study is the observation of possible age-dependent changes in the association of the ε2 and ε4 alleles with stroke, leading to a different relationship in the elderly compared with middle-aged individuals.17–19 As Kokubo and coworkers17 have pointed out, ε2 carriers tend to have an increased risk of stroke with aging, whereas ε4 carriers tend to show the opposite effect. This is in line with the epidemiological finding of positive associations between the ε4 allele and cerebral ischemia mainly in subjects <70 years of age as opposed to older individuals.

In spite of this, most studies so far have either ignored the potential effect of APOE in younger stroke patients16,19–22 or combined younger and older patients without any age strati-


**TABLE 4. 4 × 2 Table for APOE-Hypertension Interaction**

<table>
<thead>
<tr>
<th></th>
<th>Nonhypertensive With ε33 Genotype</th>
<th>Hypertensive With ε33 Genotype</th>
<th>Nonhypertensive With ε4 Genotype</th>
<th>Hypertensive With ε4 Genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cases</strong></td>
<td>70 (86.4)</td>
<td>11 (13.6)</td>
<td>25 (83.3)</td>
<td>5 (16.7)</td>
</tr>
<tr>
<td></td>
<td>105 (92.9)</td>
<td>8 (7.1)</td>
<td>19 (95.0)</td>
<td>1 (5.0)</td>
</tr>
<tr>
<td><strong>OR (95% CI)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1.84 (0.65–5.18)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>2.06 (0.79–5.38)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Cases</strong></td>
<td>80 (87.9)</td>
<td>11 (12.0)</td>
<td>26 (83.8)</td>
<td>5 (16.1)</td>
</tr>
<tr>
<td></td>
<td>117 (93.6)</td>
<td>8 (6.4)</td>
<td>20 (91.0)</td>
<td>2 (9.0)</td>
</tr>
<tr>
<td><strong>OR (95% CI)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1.93 (0.71–5.23)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>2.01 (0.77–5.22)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are number (percentage).

Synergy Index = (ORε4 carriers/ε4 carriers − ORε33 carriers/ε33 carriers)/(1 − ORε33 carriers/ε33 carriers).

Adjusted Synergy Index = (ORε4 carriers/ε4 carriers − ORε33 carriers/ε33 carriers)/(1 − ORε33 carriers/ε33 carriers).

adjSynergy Index = (ORε4 carriers/ε4 carriers − ORε33 carriers/ε33 carriers)/(1 − ORε33 carriers/ε33 carriers).

Adjusted Synergy Index = (ORε4 carriers/ε4 carriers − ORε33 carriers/ε33 carriers)/(1 − ORε33 carriers/ε33 carriers).

*Adjusted for sex, age, smoking, and cholesterol levels.

Recently, Frikke-Schmidt and coworkers\(^4\) failed to find an association in a subgroup of 63 stroke survivors <50 years of age who were recruited in the setting of an outpatient clinic. This is not consistent with our findings or with the results of a separate case-control study carried out by Chowdhury and coworkers\(^5\) supporting the hypothesis of a pathogenic link between the ε4 allele and cortical thrombosis in younger individuals. Differences in sample size and specific selection criteria are the most likely explanations for these contradictory conclusions.

Apart from the discrepant results of each separate analysis, the low frequency of the ε4 allele implies that its direct independent effect on the risk of stroke may be limited, even in younger individuals. Given the multifactorial nature of the pathophysiological process leading to cerebral ischemia, it is unlikely that the APOE polymorphism may determine a considerable increase in stroke risk per se. A single gene might increase an individual’s predisposition to conventional risk factors modulating their effects (ie, gene-environmental interaction) or interplaying with other candidate genes (ie, gene-gene interaction).\(^1\) The importance of such interactions in the pathogenesis of cerebral ischemia is emphasized by our findings. Although a positive influence of smoking on stroke risk was observed in the whole group of cases independent of the specific APOE isoforms, the magnitude of such an effect was considerably greater in subjects carrying the ε4 genotype than in subjects carrying the ε33 genotype. This is in line with the smoking-ε4 interaction observed for coronary heart disease in the second Northwick Park Heart Study (NPH-SII)\(^24\) and for preclinical carotid atherosclerosis in a subanalysis of the National Heart Lung and Blood Institute (NHLBI) Family Heart Study.\(^25\)

In vitro and animal evidence has strongly suggested a direct effect of both the APOE polymorphism and smoking on several biological pathways with relevance to vessel wall homeostasis and, in particular, on the process leading to the initiation and progression of atherosclerosis.\(^18,26\) In light of these observations, our finding of a negative association between current smoking, the ε4 allele, and the subgroup of stroke cases resulting from atherosclerosis seems somewhat contradictory. An underpowered comparison resulting from the small size of the atherosclerotic subgroup might be a likely explanation. However, the pathogenic role of additional nonatherosclerotic mechanisms in the ε4-smoking-stroke relationship cannot be ruled out a priori. The reported in vitro effect of APOE on platelet aggregability\(^28\) and lymphocyte proliferation,\(^27\) the ε4-dependent inhibition of clot lysis,\(^28\) and the involvement of this allele in other coagulation pathways\(^29\) are all in line with this hypothesis and provide plausible explanations to some recent in vivo observations.\(^30–33\) The possibility that the balance between these APOE-dependent proatherogenic and nonatherogenic mechanisms might be different in different periods of life represents an attracting and unexplored hypothesis.

**Study Limitations**

A potential shortcoming of the present study is the fact that the relatively small number of cases and the performance of multiple subgroup analyses may increase the likelihood of spurious results. As to the nonsignificant ε4-hypertension interaction, we cannot rule out the possibility that our findings might also be influenced by the short duration of hypertension as a result of the young age of the study group.

Second, categorizing smoking as current or not current may result in misclassification of exposure from differential levels of smoking among genotypes. This prevents any conclusions on a dose-dependent effect of smoking on the interaction with APOE. Finally, the recruitment of controls...
among hospital employees might theoretically introduce a selection bias because of the different background of these individuals compared with the cases. The implication of this drawback is noteworthy. However, because genotype distributions in our control group do not differ significantly from those reported in other Italian series,⁶ the possibility of a biased case-control comparison seems unlikely.

Conclusions

Overall, our findings in young adults support the concept of an independent role of the APOE e4 allele on stroke risk and suggest that an e4-smoking interaction may increase an individual’s propensity to have a cerebral ischemic event. Although further studies are needed to substantiate this hypothesis, its potential implication in future genotype-dependent primary prevention strategies should be considered.

Acknowledgments

We gratefully acknowledge Michela Cossandi and Selene Romeo, III Laboratorio di Analisi, Biotecnologie, Università degli Studi di Brescia, for technical assistance with genotyping. We also express our gratitude to all the individuals who participated in the study.

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Stroke. 2004;35:438-442; originally published online January 15, 2004;
doi: 10.1161/01.STR.0000112973.00867.98

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