Prevalence of Apolipoprotein E Phenotypes in Ischemic Cerebrovascular Disease
A Case-Control Study

Rémy Couderc, PhD; Florence Mahieux, MD; Sophie Baillleul, PhD; Gilles Fenelon, MD; Robert Mary, MD; and Jacques Fermanian, MD, PhD

Background and Purpose: Apolipoprotein E polymorphism may influence the early development of coronary artery disease. We investigated the putative role of apolipoprotein E phenotypes in cerebral infarction.

Methods: The apolipoprotein E phenotypes of 69 patients (mean ± SD age, 72 ± 11 years) who had suffered completed stroke or a transient ischemic attack and 68 sex- and age-matched control subjects free of cerebrovascular disease were determined by isoelectric focusing. The relative frequency of the apolipoprotein E phenotypes in the general population was estimated in 498 healthy blood donors (mean age, 37 years).

Results: The prevalences of hypertension, diabetes mellitus, obesity, and intermittent claudication were significantly higher in patients than in control subjects. Serum lipid and apolipoprotein B concentrations and the composition of very low density lipoproteins were not significantly different between patients and control subjects. Apolipoprotein A-I and E levels were significantly lower in patients. Cholesterol levels were higher in male patients than in male control subjects (5.10 ± 1.46 versus 4.41 ± 0.80 mmol/L; p = 0.036), and the ratio of apolipoprotein A-I to B was lower (0.77 ± 0.29 versus 1.03 ± 0.37; p < 0.001). The E3/E4 phenotype was more frequent in control subjects (85%) than in patients (72.5%; p < 0.05) and healthy blood donors (64%; p < 0.02). The E3/E2 phenotype was more frequent in patients (10.1%) than in control subjects (1.4%; p < 0.05). A stepwise logistic regression showed that the presence of stroke was significantly related to high blood pressure (p < 0.001), low apo E levels (p < 0.008), obesity (p < 0.041), the apo E phenotype (p < 0.05), and diabetes mellitus (p < 0.05).

Conclusions: The E3/E4 phenotype may protect against early vascular morbidity, and the e2 gene may be a risk factor for cerebrovascular morbidity, possibly related to diabetes, hypertension, and/or obesity.

(Stroke 1993;24:661–664)

KEY WORDS • apolipoproteins • cerebrovascular disorders • risk factors

Apolipoprotein E (apo E) is a polymorphic glycoprotein that plays a critical role in triglyceride (TG)-rich lipoprotein catabolism and cholesterol homeostasis. The genetic polymorphism of apo E results from the existence of three common codominant alleles (e2, e3, and e4) that code for three apolipoprotein isofoms, E2, E3, and E4. Apo E3 is the predominant isoform. Apo E4 differs from E3 by an amino acid substitution at position 112 (Cys→Arg) and from E2 by a substitution at position 158 (Arg→Cys). Apo E3 acts as a ligand for two receptors: the apo E or "remnant" receptor, which is specifically hepatic, and the low density lipoprotein (LDL) receptor (apo B/E receptor). The catabolism of TG-rich lipoproteins appears to be modulated by the affinity of apo E for apo E or apo B/E receptors. Apo E2 binds defectively to receptors, and this results in an increase in the number of LDL receptors, thereby lowering cholesterol levels. Apo E4 is not covalently bonded to apo A-II, and its transfer from high density lipoproteins (HDL) to TG-rich lipoproteins is enhanced. This accelerates hepatic remnant capitation by apo E receptors and downregulates the number of LDL receptors, thereby enhancing cholesterol levels. Several studies have indicated that among the three most frequent dominant alleles (e2, e3, and e4), e4 is associated with the early development of coronary artery disease and atherosclerosis.1 The role of atherosclerosis and serum lipids in the pathogenesis of cerebral infarction is unclear.2–4 Two studies on the genetic polymorphism of apo E showed a high frequency of the e4 gene, along with a low frequency of the e3 gene.5,6 However, in one of these studies the population concerned was not described, and in the other the selection of patients was very restrictive. The aim of this study was to compare the prevalence of the three most frequent alleles of apo E in a defined group of patients with ischemic cerebrovascular disease with those in a control group.

Subjects and Methods
The study group consisted of consecutive patients admitted to the neurological department. Each under-
went a complete physical examination by a senior neurologist. All had suffered an ischemic event (completed stroke or transient ischemic attack [TIA]) either a few days before their admission or in the hospital. The control subjects were free of cerebrovascular disease and were matched with the patients for sex and age on the basis of five age classes (<60, 60–69, 70–79, 80–89, and >90 years).

Vascular risk factors and associated vascular diseases were recorded in patients and control subjects and included hypertension, diabetes mellitus, obesity, current cigarette smoking, oral contraceptive use, history of migraine, ischemic heart disease, arrhythmia, and vascular claudication. Nearly all the patients underwent computed tomography of the brain, Doppler ultrasonography, cervical vascular echography, serial 12-lead electrocardiography, and two-dimensional echocardiography. Cerebral angiography and 24-hour (Holter) monitoring were performed in selected patients. The presumed causes of infarction were classified into five categories: atherosclerosis with stenosis, embolic heart disease, possible hypertensive arteriolopathy (known hypertension in the absence of another etiology), other etiologies, and undetermined etiology.

Blood was collected from all subjects after a 12-hour fast. Ethylenediaminetetraacetic acid–treated plasma and serum were stored with 0.02% sodium azide and 0.1 μmol/mL phenylmethyl sulfonyl fluoride at 4°C for no longer than 3 days. Very low density lipoproteins (VLDL) (d≤1.006 g/mL) were isolated by ultracentrifugation at 100,000g for 18 hours at 4°C. Cholesterol, TG, and phospholipids were assayed by enzymatic methods in serum and VLDL. Apo A-I, apo B, and apo E serum levels were measured by immunoturbidimetric methods. Total VLDL protein was determined using Lowry’s method in the presence of 6 mmol/L urea, with human serum albumin as standard. Apo E phenotypes were determined by direct isoelectric focusing of plasma, followed by immunoblotting with an anti–apo E antibody (DAIICHI Pure Chemical). For the purposes of this study, it was assumed that for the common isoforms the phenotype reflects the genotype.

The relative frequency of the common phenotypes of apo E in the local population was estimated in 498 healthy blood donors. Their mean age was 37.1 years, with a sex ratio (men:women) of 314:184. They were selected according to internationally accepted criteria. In particular, they were free of hypertension (blood pressure <160/100 mm Hg), diabetes, obesity, personal and family history of cardiovascular and neurovascular disease, and primary and secondary dyslipidemia.

Data are expressed as mean±SD. The χ² test, Fisher’s exact test, and Student’s t test were used for the statistical comparisons of qualitative and quantitative variables, as appropriate. A stepwise logistic regression was used to find the best model describing the relation between the dependent variable (presence or absence of ischemic cerebrovascular disease) and a set of 11 predictor variables. The 11 covariates included in the model were age, sex, hypertension, diabetes mellitus, obesity, known cardiac arrhythmia, coronary artery disease, apo A-I, apo B, and apo E levels, and apo E phenotype. We used deviance as a goodness-of-fit measure. Deviance has the same asymptotic distribution as the χ² test. A stepwise analysis of deviance table was then constructed to test for the individual contributions of the predictor variables. STATISTIX statistical software was used.

Results

The data concern 69 patients (mean age, 72.3±11.6 years; range, 49–96 years) and 68 control subjects (mean age, 72.1±11.5 years; range, 50–90 years), with sex ratios of 36:33 (men:women) and 33:35, respectively. The prevalence of risk factors and associated vascular diseases in patients and control subjects is given in Table 1.

The described event was a completed stroke in 56 cases (81.2%), TIA in 10 (14.5%), and a rapidly (3 days) regressive stroke in three (4.3%). The area involved was carotid in 57 cases (82.6%), vertebrabasilar in six (8.7%), and undetermined in six (8.7%). Thirty-one patients (44.9%) had a previous history of stroke or TIA. The main etiology was atherosclerosis with stenosis in 22 cases (31.9%), including six with associated conditions (one case of polycythemia, one of oral contraceptive use, and four of cardiac arrhythmia). Isolated embolic heart disease was found in 11.6% of the cases (seven cases of arrhythmia and one of recent myocardial infarct). A possible hypertensive arteriolopathy was presumed in 23 cases (33.3%). Four patients had other relevant etiologies (two cases of obvious polycythemia, one of obvious foramen ovale, and one of obvious megadoliche arteritis). The etiology was undetermined in 12 cases (17.4%).

Serum lipid and apo B concentrations were not significantly different in patients and control subjects (Table 2), whereas apo A-I and apo E levels were significantly lower in patients; it is noteworthy that none of these parameters was outside the normal range, and none varied significantly between the five categories of presumed causes of infarction (data not shown). The chemical composition of the VLDL isolated was similar in patients and control subjects (Table 2). In previous studies, significant differences between patients and control subjects were found when data for men and women were considered separately.9 Here, cholesterol levels were higher in male patients than in male control subjects (5.10±1.46 versus 4.41±0.80 mmol/L; p=0.036), as was the ratio of TG (mmol/L) to apo E (mg/L) (male

<table>
<thead>
<tr>
<th>TABLE 1. Prevalence of Risk Factors and Associated Vascular Diseases in Patients and Control Subjects</th>
</tr>
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<tbody>
<tr>
<td>Patients (n=69)</td>
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<tr>
<td>-----------------</td>
</tr>
<tr>
<td>Hypertension†</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
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<tr>
<td>Obesity</td>
</tr>
<tr>
<td>Current smoking (or recently stopped)</td>
</tr>
<tr>
<td>Known cardiac arrhythmia</td>
</tr>
<tr>
<td>Coronary artery disease</td>
</tr>
<tr>
<td>Intermittent claudication</td>
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</table>

Each subject could present several risk factors.

*By χ² test.
†By history and/or blood pressure ≥160/90 mm Hg.
TABLE 2. Plasma Lipid and Apolipoprotein Concentrations and Very Low Density Lipoprotein Composition

<table>
<thead>
<tr>
<th></th>
<th>Control subjects (n=68)</th>
<th>Patients (n=69)</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apo A-I (g/L)</td>
<td>1.09±0.22</td>
<td>0.92±0.27</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Apo B (g/L)</td>
<td>1.16±0.33</td>
<td>1.05±0.24</td>
<td>NS</td>
</tr>
<tr>
<td>Apo E (mg/L)</td>
<td>57±19</td>
<td>45±20</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>TG (mmol/L)</td>
<td>1.15±0.44</td>
<td>1.28±0.79</td>
<td>NS</td>
</tr>
<tr>
<td>PL (mmol/L)</td>
<td>2.55±0.52</td>
<td>2.59±0.47</td>
<td>NS</td>
</tr>
<tr>
<td>Cholesterol (mmol/L)</td>
<td>4.96±1.10</td>
<td>5.03±1.24</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are mean±SD. Apo, apolipoprotein; TG, triglycerides; PL, phospholipids; VLDL, very low density lipoprotein; TP, total proteins.

*pBy Student's t test.

patients, 0.035±0.023 versus male control subjects, 0.022±0.009; p=0.003), since apo E levels were not significantly different. Moreover, the ratio of apo A-I (g/L) to apo B (g/L) was significantly lower in patients than in control subjects (0.77±0.29 versus 1.03±0.37; p<0.001). Apo E levels (mg/L) were significantly lower in female patients than in the corresponding control subjects (46±16 versus 60±20; p=0.002). However, the ratio of TG to apo E was higher in female patients than in female control subjects (0.028±0.013 versus 0.020±0.007; p=0.002). The relative frequencies of apo E phenotypes were significantly different in patients, control subjects, and healthy blood donors (Table 3). The E3/E3 phenotype was more frequent in control subjects (85%) than in patients (72.5%) (p<0.05) and more frequent in patients than in healthy blood donors (64%) (p<0.02). The E3/E2 phenotype was more frequent in patients than in control subjects (p<0.05). Patients carrying the e4 allele had higher levels of plasma cholesterol and apo B than those with the E3/E3 or E3/E2 phenotypes. Neither the concentration of other lipids or apolipoproteins nor the VLDL composition varied with the apo E phenotype. Subgroups of presumed causes of cerebral infarction were too small for statistical analysis. However, the highest frequency of E4/E3 phenotype was encountered in the arteriolopathy subgroup (20.8%), and the E3/E2 phenotype was most frequent in the embolic-genic heart disease subgroup (18.2%).

The stepwise logistic regression analysis indicated that hypertension (p<0.0001), apo E level (p<0.008), obesity (p<0.041), the apo E phenotype (E3/E3 versus the others) (p<0.05), and diabetes mellitus (p<0.05) were significant and independent predictors of the presence of ischemic cerebrovascular disease (Table 4).

**Discussion**

The population studied here offers a good cross section of elderly people exposed to ischemic stroke. The mean age of our sample was 10 years higher than that of the largest groups previously studied.10-13 Because of this, it is overrepresented in women14,15 and, as a result, the significance of obesity as a risk factor is augmented. Given these slight discrepancies and the known difficulties in interrater comparisons among different classifications,15,16 the distribution of clinical data and risk factors in our sample does not appear to differ notably from that in previous studies.

Numerous authors have assessed the relation between carotid artery atherosclerosis and various lipids or lipoproteins. However, the differences in study design make the comparison of data difficult.2 Apo A-I has only been measured in a few studies.3,4,8,9,17 A significant negative association has been found between ischemic cerebrovascular disease and apo A-I levels in a study of 30 patients with familial hypercholesterolemia. The decrease in apo A-I plasma levels in the patients in our study confirms these data, although it was larger than

TABLE 3. Prevalence of Apolipoprotein E Phenotypes in Ischemic Cerebrovascular Patients, Control Subjects, and Healthy Blood Donors

<table>
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<tbody>
<tr>
<td>Age (years)</td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>Patients 72.3±11.6</td>
<td>50</td>
<td>72.5*</td>
<td>10</td>
<td>14.5</td>
<td>7</td>
<td>10.1*</td>
</tr>
<tr>
<td>Control subjects 72.1±11.5</td>
<td>58</td>
<td>85†</td>
<td>9</td>
<td>13.2†</td>
<td>1</td>
<td>1.4†</td>
</tr>
<tr>
<td>HBD 37.1±10</td>
<td>319</td>
<td>64</td>
<td>100</td>
<td>20.1</td>
<td>59</td>
<td>11.9</td>
</tr>
</tbody>
</table>

Age values are mean±SD. HBD, healthy blood donors.

*p<0.05 different from control subjects by Fisher's exact test.

†p<0.02 different from HBD by χ² test.
previously reported. However, this parameter depended on the presence of diabetes ($p < 0.01$).

Because no differences in the ratio of HDL cholesterol to apo A-I or in TG levels were found (data not shown), a preferential redistribution of cholesterol from HDL to accumulated VLDL hypothesized by Matsuda et al. would not explain our results. However, the significant increase in the ratio of TG to apo E in patients suggests that TG-rich lipoprotein catabolism is altered, which is in agreement with a recent report of an increase in intermediate density lipoproteins in men with ischemic stroke.6

The E3/E3 phenotype was less frequent in our patients than in the control subjects, confirming previously reported data.5 However, in these two studies a higher prevalence of E4 polymorphism was found in patients than in control subjects. This difference between these studies and the present study could be due to patient selection criteria. Indeed, only men without coronary heart disease, vasculitis, diabetes mellitus, and other endocrine, hepatic, and renal diseases were included in one, and the population was not described in the other.5 Moreover, no clear relation between apo E polymorphism and plasma lipid levels was found in the larger study, contrary to most population studies.1

A stepwise logistic regression analysis was performed to remove possible bias due to unmatched factors and to appreciate the influence of covariables. In addition to the matching variables (age and sex), each of the variables strongly associated with the presence of ischemic cerebrovascular disease was included in the model. Plasma apo B and total cholesterol levels were strongly correlated ($r = 0.816$). The latter parameter was therefore not included in the regression model. Plasma TG levels, which depended on the presence of diabetes, were also excluded. It is noteworthy that apo E plasma levels and the apo E phenotype were the sole significant lipidic predictors of ischemic cerebrovascular disease. However, an age- and sex-matched control group differed significantly from the “normal” population of healthy young blood donors in terms of apo E phenotype frequency. This difference in apo E phenotype frequencies between the control and blood donor groups could be due to an insufficient number of control subjects. Another possible explanation is that the E3/E3 phenotype may act as a protective factor against early vascular morbidity. In other words, the presence of the e2 allele, calculated from phenotype frequencies (healthy blood donors, 7.2%; patients, 6.5%; control subjects, 0.7%), leads to increased morbidity at an age younger than the mean age of our control subjects. This may be due to a potentialization of other vascular risk factors rather than to a direct deleterious effect, as suggested by studies showing an overrepresentation of the E2 isoform associated with lower limb atheromatosis, even in the absence of dyslipidemia. 18

References
R Couderc, F Mahieux, S Bailleul, G Fenelon, R Mary and J Fermanian

Stroke. 1993;24:661-664
doi: 10.1161/01.STR.24.5.661

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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