Apolipoprotein E Genotype Is Related to Progression of White Matter Lesion Load

Ophélie Godin, MSc; Christophe Tzourio, MD, PhD; Pauline Maillard, PhD; Annick Alpérovitch, MD, MSc; Bernard Mazoyer, MD, PhD; Carole Dufouil, PhD

Background and Purpose—The relationship between white matter lesions (WMLs) and the apolipoprotein E genotype has been controversial from cross-sectional studies and no longitudinal finding has been reported. We investigated whether the apolipoprotein E genotype influences baseline and evolution over 4-year follow-up of WML volumes in a population-based sample of 1779 non-demented subjects aged 65 to 80 years old at enrollment.

Methods—The sample consisted of 3C-Dijon study participants who had 2 cerebral MRIs, at entry and at 4-year follow-up. WML volumes were estimated using a fully automatic procedure. We performed analysis of covariance to evaluate the relationship between apolipoprotein E genotype and WML load and progression.

Results—Multivariable analyses showed that e4/e4 individuals had both significantly higher WML volume at baseline and higher WML increase over 4-year follow-up than noncarriers and heterozygous of the e4 allele for apolipoprotein E genotype.

Conclusion—These findings suggest it might be important to take into account WML severity when assessing the relationship between apolipoprotein E and dementia. (Stroke. 2009;40:3186-3190.)

Key Words: ApoE ■ cerebrovascular ■ elderly ■ epidemiology ■ MRI

Epidemiological studies have shown that the apolipoprotein E (ApoE) genotype is a major genetic risk factor for Alzheimer disease (AD).1 The underlying mechanisms remain incompletely understood. Both animal and neuropathological studies suggest that the ApoE-e4 allele enhances brain amyloid beta (Aβ) deposition, which induces amyloid plaques formation.2-4 At a macroscopic level, the impact of ApoE on brain atrophy in both AD and healthy elderly has been reported.5 Similar to the ApoE genotype, results from recent epidemiological studies emphasize the predictive value of white matter lesions (WMLs) load on the risk of AD.6 However, relatively little is known about how ApoE and WML load are interrelated.

Reports on the association between WML severity and ApoE are controversial, some cross-sectional studies showing no association7-8 and others reporting a positive association between the presence of e4 allele and greater WML volumes.9,10 There has been, to date, no longitudinal study exploring the association between ApoE genotype and WML change in non-demented people.

In a large population-based cohort of elderly, free of dementia at enrollment, who had a cerebral MRI at both study entry and 4-year follow-up, we assessed, cross-sectionally and longitudinally, the relationship between ApoE genotype and WML volumes.

Materials and Methods

Study Population

The Three-City (3C) Study is a multicenter population-based cohort study conducted in 3 French cities (Bordeaux, Dijon, and Montpellier) and designed to estimate the risk of dementia and cognitive impairment attributable to vascular factors as described elsewhere.11 The protocol was approved by the Ethical Committee of the University Hospital of Kremlin-Bicêtre. Between March 1999 and March 2001, 9293 noninstitutionalized individuals aged ≥65 years, selected from the electoral rolls of the 3 cities, agreed to participate in this project. Each participant signed an informed consent and was followed-up every 2 years during 4 years. In Dijon (n=4931), a cerebral MRI examination was proposed to those aged 65 to 80 years old who were enrolled between June 1999 and September 2000 (N=2763). Although 2285 subjects (82.7%) agreed to participate, because of financial limitations, 1779 examinations were performed and interpretable. Compared to the rest of the 3C-Dijon participants, those who had both valid MRI and DNA available (N=1779) were on average younger (72.4 years; SD, 4.1) versus 75.9 years (SD,6.1; P<0.0001), and the proportion of participants carrying e4 alleles did not differ (1.0% versus 1.2%; P=0.24). After 4-year follow-up, 1319 subjects had a second valid MRI. Subjects without follow-up MRI (N=460, among whom 54 died before the second MRI...
WML volumes were calculated by summing the volumes of all the lesions detected in each area. Otherwise, it was labeled as deep. Periventricular WML and deep WML were categorized with the help of voxel-based techniques, total intracranial volume (TIV) was computed as the sum of the gray matter, white matter, and cerebrospinal fluid volumes.

**Covariates**
Selected covariates were among those measured at study entry. Education level was defined in 4 categories ranging from primary certificate level (low) to baccalaureate or university degree (high). Subjects were considered as hypertensive if systolic blood pressure was ≥160 mm Hg or diastolic blood pressure was ≥95 mm Hg or if they were on antihypertensive medication. Hyperglycemia was defined as 6.1 mmol/L ≤ glycemia < 7 mmol/L or diabetes (glycemia ≥ 7 mmol/L).

**Statistical Analysis**
Cross-sectional and longitudinal analyses were respectively based on samples of 1779 and 1319 subjects. The relationship between baseline WML volume and ApoE genotype was computed using analysis of covariance adjusting for sex, age, education level, and total intracranial volume (Model 1). We performed analysis of covariance adjusted for sex, age, education level, and body mass index was computed. We used SAS (Release 9.1; SAS Statistical Institute, Cary, NC) for the analyses.

**Results**
Principal characteristics of the participants and factors associated with ApoE genotype are displayed in Table 1. Subjects’ mean age was 72.4 years (SD, 4.1) and 60.5% were women. There were no significant differences between number of ε4 alleles for ApoE genotype and demographic status or vascular risk factors frequencies.

### Table 1. Characteristics of Participants

<table>
<thead>
<tr>
<th></th>
<th>Total (n=1779)</th>
<th>None (n=1383)</th>
<th>One (n=375)</th>
<th>Two (n=21)</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Female sex, %</strong></td>
<td>60.5</td>
<td>61.4</td>
<td>57.3</td>
<td>61.9</td>
<td>0.36</td>
</tr>
<tr>
<td><strong>Mean age, years (SE)</strong></td>
<td>72.4 (4.1)</td>
<td>72.5 (0.1)</td>
<td>72.2 (0.2)</td>
<td>71.2 (0.9)</td>
<td>0.17</td>
</tr>
<tr>
<td><strong>Education level, %</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>16.2</td>
<td>16.4</td>
<td>15.5</td>
<td>14.3</td>
<td>0.35</td>
</tr>
<tr>
<td>Medium-low</td>
<td>44.1</td>
<td>44.9</td>
<td>42.1</td>
<td>23.8</td>
<td></td>
</tr>
<tr>
<td>Medium-high</td>
<td>18.9</td>
<td>18.6</td>
<td>19.5</td>
<td>28.6</td>
<td></td>
</tr>
<tr>
<td>High</td>
<td>20.8</td>
<td>20.1</td>
<td>22.9</td>
<td>33.3</td>
<td></td>
</tr>
<tr>
<td><strong>Hypertension†, %</strong></td>
<td>59.6</td>
<td>60.5</td>
<td>56.3</td>
<td>66.7</td>
<td>0.28</td>
</tr>
<tr>
<td><strong>Hyperglycemia or diabetes‡, %</strong></td>
<td>8.0</td>
<td>7.7</td>
<td>9.3</td>
<td>4.8</td>
<td>0.51</td>
</tr>
<tr>
<td><strong>History of cardiovascular disease‡, %</strong></td>
<td>11.3</td>
<td>11.7</td>
<td>10.4</td>
<td>5.0</td>
<td>0.53</td>
</tr>
</tbody>
</table>

*Analysis of covariance adjusted for age and sex.
†Self-reported history of stroke, arteritis, and myocardial infarction.
‡Defined as 6.1 mmol/L ≤ glycemia < 7 mmol/L, or diabetes (glycemia ≥ 7 mmol/L).
Cross-Sectional Findings

After adjusting for age, sex, education level, and TIV, baseline total WML volume was significantly higher in subjects who were homozygous for the ApoE e4 allele compared with the others (Table 2). This finding was consistent for both periventricular and deep WML volumes. Adjustment for additional potential confounders (Model 2, Table 2) led to similar findings. There was no trend for a e4 allele dose-dependent association with total WML volume ($P=0.51$).

Longitudinal Findings

Over 4-year follow-up, mean increase in total WML volume was 1.4 cm$^3$ (SD, 2.8) in the entire sample. It differed significantly by ApoE genotype, subjects who were e4/e4 having higher increase in WML volume (Table 3). Carriers of e4/e4 had an increase on average 1.8 cm$^3$ higher in periventricular WML volume and 0.30 cm$^3$ higher in deep WML volume than the rest of the participants. Further adjustments for vascular factors did not modify the findings for total WML or periventricular WML volumes (Model 2, Table 3). For deep WML volumes, after full adjustment, the probability value was 0.40.

In multivariable models (Model 2), test for a e4 allele dose-dependent association almost reached statistical significance for total WML volume increase ($P=0.07$) and periventricular WML volume increase ($P=0.08$).

Stratified analyses by sex, age, or vascular risk factors were not in favor of any significant interaction or even trend (data not shown).

Discussion

In a large population-based sample of elderly, we found from both cross-sectional and longitudinal analyses the significant impact of ApoE genotype on WML load. Indeed, subjects who were e4 homozygous for ApoE genotype had on average significantly higher WML volumes at baseline than e4 heterozygous or e4 allele noncarriers (cross-sectional analyses) but also exhibited higher WML increases over 4-year follow-up compared with the others. A similar pattern of associations was observed whatever the WML localization (periventricular or deep). Controlling for vascular factors (hypertension, diabetes, history of cardiovascular disease, alcohol and tobacco consumptions, body mass index, and TIV) did not affect the overall findings.

Previous reports on the association between ApoE genotype and WML load have provided inconsistent results. Most of them have concluded there was no association, whereas other studies, mainly in nondemented individuals, have reported a positive association. However, it should be noted that these studies have compared e4 noncarriers with individuals carrying at least one e4 allele, which does not

Table 2. Cross-Sectional Association Between WML Volume at Study Entry and ApoE Genotype

<table>
<thead>
<tr>
<th>No. of e4 alleles for the ApoE genotype</th>
<th>Total WMLs Mean Volume, cm$^3$ (SE)</th>
<th>Periventricular WMLs Mean Volume, cm$^3$ (SE)</th>
<th>Deep WMLs Mean Volume, cm$^3$ (SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N Model 1† Model 2‡</td>
<td>Model 1* Model 2†</td>
<td>Model 1* Model 2†</td>
</tr>
<tr>
<td>None</td>
<td>1383 5.64 (0.14) 6.21 (0.28)</td>
<td>4.38 (0.12) 4.85 (0.25)</td>
<td>1.27 (0.03) 1.35 (0.05)</td>
</tr>
<tr>
<td>One</td>
<td>375 5.41 (0.25) 5.94 (0.35)</td>
<td>4.19 (0.22) 4.65 (0.31)</td>
<td>1.22 (0.05) 1.29 (0.07)</td>
</tr>
<tr>
<td>Two</td>
<td>21 9.14 (1.05) 9.62 (1.12)</td>
<td>7.23 (0.94) 7.83 (0.99)</td>
<td>1.91 (0.20) 1.78 (0.22)</td>
</tr>
</tbody>
</table>

|$P$ value$^{2df}$ 0.003 0.004 0.007 0.005 0.004 0.06

*Standard error.
†Analysis of covariance adjusted for age, sex, education level, and TIV.
‡Analysis of covariance adjusted for age, sex, education level, hypertension, diabetes, history of cardiovascular disease, alcohol and tobacco consumptions, body mass index, and TIV.

Table 3. Longitudinal Association Between WML Volume Change Over 4-Year Follow-Up and ApoE Genotype

<table>
<thead>
<tr>
<th>No. of e4 alleles for the ApoE genotype</th>
<th>Total WMLs Mean Volume Change,* cm$^3$ (SE)</th>
<th>Periventricular WMLs Mean Volume Change, cm$^3$ (SE)</th>
<th>Deep WMLs Mean Volume Change, cm$^3$ (SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N Model 1† Model 2§</td>
<td>Model 1* Model 2†</td>
<td>Model 1* Model 2†</td>
</tr>
<tr>
<td>None</td>
<td>1030 +1.32 (0.09) +1.34 (0.19)</td>
<td>+0.97 (0.08) +1.09 (0.17)</td>
<td>+0.05 (0.02) +0.008 (0.04)</td>
</tr>
<tr>
<td>One</td>
<td>275 +1.48 (0.17) +1.49 (0.23)</td>
<td>+1.09 (0.15) +1.20 (0.21)</td>
<td>+0.08 (0.03) +0.04 (0.05)</td>
</tr>
<tr>
<td>Two</td>
<td>14 +3.46 (0.72) +3.31 (0.76)</td>
<td>+2.78 (0.65) +2.85 (0.69)</td>
<td>+0.35 (0.15) +0.17 (0.16)</td>
</tr>
</tbody>
</table>

|$P$ value$^{2df}$ 0.01 0.02 0.02 0.12 0.40

*Change measured as difference in WML volumes between 4-year follow-up and baseline.
†Standard error.
‡Analysis of covariance adjusted for age, sex, education level, time between the two MRI examination, and TIV.
§Analysis of covariance adjusted for age, sex, education level, time between the two MRI examinations, WML load at entry, hypertension, diabetes, history of cardiovascular disease, alcohol and tobacco consumptions, body mass index, and TIV.
allow comparisons with our findings. Only one study, in a sample of 60 patients with AD, also reported that e4e4 carriers had significantly more extensive WMLs than e4e3 and e3e3 carriers.15

Our cross-sectional data are not in favor of a dose-dependent effect between number of e4 alleles carried and lesion load, whereas in longitudinal data analysis, a trend close to statistical significance in favor of a dose-dependent effect is observed mainly for increase in total and periventricular WML volumes. One hypothesis could be that with the 3C participants being very healthy, the association between ApoE genotype and higher WML volume is not yet visible among e4 heterozygous, hence the small trend in longitudinal analysis. We have investigated this hypothesis further by splitting the e4 heterozygous group according to the median age, but the results did not reveal a difference in WML load between the 2 age groups either in cross-sectional or in longitudinal analysis. Another hypothesis is that carrying one allele other than e4 might be enough to slow the process leading to WML progression.

ApoE genotype being a well-established risk factor for dementia and WML being also related to dementia risk, it could be argued that incipient AD in e4e4 carriers explains our findings. We have explored that assumption in 2 ways; first, we have excluded the 7 subjects who had converted to dementia after baseline examination and second, we have rerun the analyses on the subsample of subjects having Mini Mental State Examination score >24 at baseline. In both scenarios, results were unchanged so we can exclude the hypothesis that our findings are driven by dementia. Some limitations of our study should be considered; our findings are based on a relatively small number of e4e4 carriers that require being cautious in interpreting and generalizing the results.

The strengths of our study include the sample size, the population-based setting, the repeated cerebral MRI, and the fully automated quantification of WML volume using a validated and reproducible algorithm.16

This study is the first showing, both longitudinally and cross-sectionally, the relationship between WML load and ApoE genotype in a population-based sample. Various mechanisms could be considered to explain this association. The association between e4e4 and increased WML volumes could reflect cerebral blood flow reduction. Indeed, on the one hand, cerebral blood flow has been found to be decreased in subjects who are homozygous for the e4 allele,17 and, on the other hand, there are arguments showing that cerebral blood flow might play a role in the occurrence of WML.18 It has also been suggested, from a molecular perspective, that the presence of the apolipoprotein e4 allele may cause an increased vulnerability to slight chronic hypoperfusion of the white matter by reducing the range of mechanical and chemical flexibility of the glial cytoskeleton.19

However, the most likely scenario linking WML load to the ApoE genotype is through cerebral amyloid angiopathy (CAA). CAA is caused by the deposition of amyloid within the media and adventitia of small- to medium-sized cerebral arteries, which may lead to vessel fragility. It has been shown that the e4 allele promotes vascular deposition of the β-amyloid peptide and therefore CAA,20 and vascular amyloid deposition may alter white matter perfusion through vascular stenosis dysfunction.21 Therefore, one could hypothesize that CAA is an intermediate factor in the association between ApoE and WML severity. It could also be hypothesized that WML is intermediate in the association between ApoE genotype and CAA, although no biological basis for such a pathway is currently available. If the hypothesis that CAA is an intermediate factor in the association between ApoE and WML severity is true, this could have important consequences to elucidate more globally the link between WML and dementia, WML being potentially a marker of CAA disease activity.

In a broader sense, WML load could be considered as a surrogate marker of both CAA disease activity and dementia risk in future clinical trials.

Sources of Funding
The 3-City Study is conducted under a partnership agreement among the Institut National de la Santé et de la Recherche Médicale (INSERM), the Victor Segalen–Bordeaux II University, and Sanofi-Aventis. The Fondation pour la Recherche Médicale funded the preparation and initiation of the study. The 3C Study is also supported by the Caisse Nationale Maladie des Travailleurs Salariés, Direction Générale de la Santé, MGEN, Institut de la Longévité, Conseils Régionaux of Aquitaine and Bourgogne, Fondation de France, and Ministry of Research–INSERM Programme “Cohortes et collections de données biologiques.” C.T. has received investigator-initiated research funding from the French National Research Agency (ANR).

Disclosures
O.G. and C.D. have received consulting fees from EISAI. C.T. has received fees from Sanofi-Synthelabo for participation in a data safety monitoring board and from Merck-Sharp & Dohme for participation in a scientific committee.

References


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Stroke. 2009;40:3186-3190; originally published online July 30, 2009;
doi: 10.1161/STROKEAHA.109.555839
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

The online version of this article, along with updated information and services, is located on the
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