Interaction Between Hypertension, apoE, and Cerebral White Matter Lesions

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Background and Purpose—Cerebral white matter lesions (WMLs) are frequently found on magnetic resonance imaging scans in both cognitively intact and demented elderly persons. Vascular risk factors, especially hypertension, are related to their presence. However, not every person with vascular risk factors has WMLs, which suggests interaction with other determinants, eg, genetic factors. The ε4 allele of the apolipoprotein E gene (apoE) may be a candidate because this allele is associated with both the vascular risk factors and the consequences (cognitive impairment, dementia) of WMLs.

Methods—We investigated apoE genotype, blood pressure levels, and their interaction in relation to subcortical and periventricular WMLs in 971 participants in the Rotterdam Scan Study.

Results—ApoE ε4 carriers had a significantly higher subcortical WML volume than did apoE ε3ε3 carriers (adjusted mean difference, 0.5; 95% confidence interval, 0.2 to 0.8), irrespective of hypertension. This was not found for periventricular WMLs. Participants with both hypertension and at least 1 apoE ε4 allele had the highest degree of both types of WML; the interaction was statistically significant for subcortical WMLs (P=0.016).

Conclusions—apoE ε4 carriers are at increased risk for WMLs if they suffer from hypertension as well. This may reflect a diminished capacity for neuronal repair in apoE ε4 carriers. (Stroke. 2004;35:1057-1062.)

Key Words: epidemiology ■ hypertension ■ cerebrovascular disease ■ MRI ■ other arteriosclerosis

Cerebral white matter lesions (WMLs) are frequently found on magnetic resonance imaging (MRI) scans in both cognitively intact and demented elderly persons. WMLs are related to both cognitive impairment and dementia. Pathologically, WMLs are characterized by arteriosclerosis, myelin loss, and gliosis. Vascular risk factors, especially hypertension, are related to the presence of these lesions. That not every person with vascular risk factors has cerebral WMLs suggests that the occurrence of WMLs is dependent on the interaction between vascular risk factors and other factors, eg, genotype. Support for genetic involvement in the development of WMLs comes from a twin study, in which the volume of WMLs was more strongly correlated for monozygotic than for dizygotic twins.

A possible candidate for such a genetic factor might be the apolipoprotein E (apoE) ε4 allele, 1 of the 3 polymorphic forms of the apoE gene, because this allele is associated not only with the vascular risk factors for WMLs but also with its consequences, particularly cognitive impairment and dementia. The apoE gene encodes apoE, which has important functions in lipid metabolism and neuronal repair. Initially, it was hypothesized that the ε4 allele was related to cognitive impairment, mainly because of the presence of WMLs. However, previous studies found no relation between an ε4 allele and WMLs, neither in patients with Alzheimer’s disease or vascular dementia nor in population-based studies. On the other hand, a twin study showed that the presence of an ε4 allele in combination with unspecified cardiovascular or cerebrovascular disease did increase the risk for WMLs. Because hypertension is the primary risk factor for vascular disease and WMLs, we hypothesized that the destructive effect of vascular risk factors, in particular hypertension, on the white matter might be enhanced by the presence of an ε4 allele. We investigated this in the population-based Rotterdam Scan Study.

Methods

Study Population

The Rotterdam Scan Study investigated the determinants and cognitive consequences of age-related brain abnormalities in the elderly. In 1995 to 1996, 1904 subjects aged between 60 and 90 years were randomly selected by strata of age (5 years) and sex from 2 large ongoing, prospective, follow-up studies, the Zoetermeer Study and the Rotterdam Study. Both studies have been described in detail elsewhere. In brief, the Zoetermeer Study is a prospective,
population-based study of 10,361 subjects aged 5 to 91 years at baseline; its aim is to study the determinants of chronic disease. The Rotterdam Study is a prospective, population-based cohort study of 79,873 subjects aged 55 years and older that investigates the determinants of neurologic, cardiovascular, endocrine, and ophthalmologic diseases in the elderly. The populations in both studies are almost completely white and of Dutch origin.

For the Rotterdam Scan Study, subjects were invited to participate by letter and subsequently contacted by telephone. Upon agreement to participate, a list of contraindications was reviewed to assess eligibility: dementia, blindness, or the presence of MRI contraindications, such as prosthetic valves, pacemakers, cerebral aneurysm clips, a history of intraocular metal fragments, cochlear implants, and claustrophobia. Of 1,904 invited subjects, 1,717 were eligible. The total response rate was 63%. The Rotterdam Study had 563 respondents, and the Zoetermeer Study had 514 respondents. For the 1,077 individuals, blood pressure measurements and a cerebral MRI scan were available; for 971, apoE genotype data were also available; this article reports on these 971 participants. Those without apoE genotype data did not significantly differ from our study population, except for a higher body mass index (BMI). Each participant signed an informed consent form. The study was approved by the medical ethics committee of the Erasmus University, Rotterdam, The Netherlands.

Hypertension
Hypertension was defined as systolic blood pressure $\geq 160$ mm Hg, diastolic blood pressure $\geq 95$ mm Hg, self-reported use of blood pressure–lowering medication, or any combination of these parameters. Information on blood pressure–lowering medication was obtained from a computerized, structured questionnaire, which was checked by a physician.

ApoE Genotyping
ApoE genotyping was performed as described previously on coded genomic DNA samples, without knowledge of the WML rating. The results were read by 3 independent raters; in cases of discrepancies, the apoE genotyping was repeated.

MRI Scanning Protocol
For all participants, an axial T1-, T2-, and proton density–weighted cerebral MRI scan was performed on a 1.5-T MRI device. Subjects recruited from the Zoetermeer Study were scanned with a 1.5-T MR gyrosan (Philips), and participants from the Rotterdam Study were scanned with a 1.5-T MR Vision device (Siemens). Slice thickness was 6 mm and 5 mm, respectively, with an interslice gap of 20.0%. The images were printed on hard copy with a reduction factor of 2.7.

WML Rating Scale
WMLs were considered present if they appeared hyperintense on both the proton density– and T2-weighted images without hypointensity on the T1-weighted images. WMLs were rated separately for subcortical and periventricular regions. WMLs in the basal ganglia or infratentorial regions were not taken into account. When there was an asymmetry between left- and right-sided periventricular WMLs, the score of the more severely affected side was used in the analysis. The number and size of subcortical WMLs were rated in both hemispheres on hard copy according to the largest diameter of a lesion in any of the slices in which the lesion could be observed in categories of small (cutoff 3 mm; diameter, 1 mm), medium (3 to 10 mm; diameter, 6 mm), or large (>10 mm; diameter, 12 mm) lesions. Confluent lesions were considered to be large, subcortical WMLs. To calculate the volume of subcortical WMLs on hard copy, they were considered to be spherical with a predefined diameter per size category.

Periventricular WMLs were rated semiquantitatively per region: adjacent to the frontal horns, to the lateral wall of lateral ventricles, and to the occipital horns, on a scale ranging from 0 to 3. The overall degree of periventricular WMLs was calculated by adding the scores for the 3 separate categories (range, 0 to 9). All MRI scans were examined by 2 individuals from a pool of experienced raters. Interrater and intrarater weighted kappa values for periventricular WMLs were 0.73 and 0.88, respectively. The weighted kappa was obtained by giving weights to the frequencies in each cell of the table according to the distance from the diagonal that indicates agreement. Thus, we give cells on the diagonal a weight of 1. For the periventricular WML score of 0 to 3, weights for discrepancies of 0, 1, 2, and 3 are thus 1, 2/3, 1/3, and 0, respectively. For total subcortical WML volume, the interrater and intrarater intraclass correlation coefficients were 0.88 and 0.95, respectively.

Measurement of Other Covariates
Height and weight of participants were measured without shoes in light clothing. The BMI was calculated as weight (kilograms) divided by height squared (meters, squared). Blood pressure was measured twice on the right arm with a random-zero sphygmomanometer with the participant in a sitting position. The average of these 2 measurements was used. As an indicator of atherosclerosis, the ankle–brachial index was calculated by the measurement of blood pressure of the tibial artery with an 8-MHz continuous-wave Doppler probe (Huntleigh 500D, Huntleigh Technology). For the brachial artery, the blood pressure was measured with a random-zero sphygmanometer with the participant in a supine position. The ankle–brachial index was defined by the averaged systolic blood pressure at the left and right posterior tibial artery divided by the systolic pressure of the right arm. Subjects with an ankle–brachial index <0.9 were considered to have peripheral arterial disease. Diabetes mellitus was considered present if the participant was taking oral antidiabetic medication, insulin, or if the random or postload glucose level was $>11.1$ mmol/L.

Statistical Analysis
The mean volume of subcortical WMLs and the mean grade of periventricular WMLs were calculated by ANCOVA for participants with or without hypertension, irrespective of the apoE genotype. This was also done for apoE 4 carriers (apoE 4/4 and apoE 4/4) compared with apoE 3/3 homozygotes as the reference group, irrespective of hypertension. apoE 4/4 (n=22), apoE 3/3 (n=116), and apoE 3/2 (n=4) genotypes were excluded from these analyses.

To investigate the interaction between hypertension and apoE with respect to WMLs, the severity of WMLs was compared across 4 groups through linear regression analysis: (1) no hypertension and apoE 3/3 genotype (reference group); (2) hypertension and apoE 3/3 genotype; (3) no hypertension and apoE 4/4 carrier; and (4) hypertension and apoE 4/4 carrier. Interaction between hypertension and apoE was also tested by adding the interaction term to a model that contained variables for hypertension (yes/no) and apoE 4 carrier status (yes/no).

In all analyses, adjustments were made for possible confounding factors, including age, sex, and intermediate vascular risk factors, such as BMI, peripheral arterial disease, diabetes mellitus, and study site (Zoetermeer or Rotterdam).

Results
Genetic data on the apoE polymorphism were available for 971 subjects. The e2, e3, and e4 allele frequencies were 0.075, 0.767, and 0.158, respectively. The distribution of the apoE genotypes was in Hardy-Weinberg equilibrium.

For all subjects, the mean degree of periventricular WMLs was 2.4 (SD, 2.2; range, 0 to 9), and the mean volume of subcortical WMLs was 1.3 mL (SD, 2.8 mL; range, 0 to 29.5 mL). There were no significant differences between the 2 groups except for age (Table 1). Subjects with an apoE 4/4 allele were slightly younger than apoE 3/3 genotypes (71.1 vs 72.6 years of age; P=0.0056). About one half of all participants had hypertension. Of all participants, $\approx20\%$ and
periventricular regions than did normotensives, irrespective of their apoE genotype. ApoE ε4 carriers had a higher subcortical WML volume than did subjects with the ε3ε3 genotype, but this was not found for periventricular WMLs. There was a significant interaction between hypertension and the apoE ε4 allele with regard to subcortical but not periventricular WMLs.

A strength of this study is its large number of elderly subjects from the general population, including institutionalized persons. Although our study was population-based, some selection bias may have occurred by selective nonresponse. Probably the participation rate was lowest among subjects with the highest degree of WMLs, because they are the most likely to forget appointments at the study center because of memory problems or to have difficulties in reaching the study center because of gait disturbances related to WMLs. Another form of selection bias may be underrepresentation of ε4 carriers by exclusion of demented patients, who have the highest odds of having an ε4 allele. A third potential source of error is survival bias by reduced survival in patients with the highest degree of WMLs, because they are the most likely to forget appointments at the study center because of memory problems or to have difficulties in reaching the study center because of gait disturbances related to WMLs. Addition of the distribution of apoE genotypes was in Hardy-Weinberg equilibrium in our population. Therefore, we consider it unlikely that either selection bias or survival bias influenced our findings.

Our definition of hypertension was chosen to increase the contrast between hypertensives and nonhypertensives. By the application of current guidelines of hypertension, more people would qualify as hypertensive than according to our definition. Consequently, the hypertensive group would encompass more people with a relatively mild degree of WMLs. This would lead to a “dilution” of the difference in the degree of WMLs between the normotensive and hypertensive groups.

Like other smaller studies, we found an association only between the apoE ε4 allele and subcortical WMLs, irrespective of the presence of hypertension. A possible explanation for this observation may be a difference in the vascularization between subcortical and periventricular white matter, the latter being an arterial border zone that is more susceptible to a decrease in cerebral blood flow than the subcortical white matter. Compatible with this view is the presence of severe periventricular WMLs in patients with reduced cerebral blood flow associated with longstanding hypertension. In this marginally perfused periventricular area, WMLs may readily emerge, especially in the presence of chronic hypertension, whereas other vascular risk factors also predominantly seem to affect the periventricular white matter in contrast to the subcortical white matter.

### Discussion

We found that participants with hypertension had a significantly higher degree of WMLs in both subcortical and periventricular regions than did normotensives, irrespective of their apoE genotype. ApoE ε4 carriers had a higher subcortical WML volume than did subjects with the ε3ε3 genotype, but this was not found for periventricular WMLs. There was a significant interaction between hypertension and the apoE ε4 allele with regard to subcortical but not periventricular WMLs.

### Table 1. Characteristics of the Study Population by apoE Status

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>apoE ε3ε3</th>
<th>apoE ε4+</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of subjects</td>
<td>568</td>
<td>261</td>
</tr>
<tr>
<td>Women, %</td>
<td>50.9</td>
<td>51.7</td>
</tr>
<tr>
<td>Mean age, y</td>
<td>72.6 (7.4)</td>
<td>71.1 (7.1)†</td>
</tr>
<tr>
<td>Hypertension, %</td>
<td>52.1</td>
<td>50.2</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>147.6 (21.5)</td>
<td>148.2 (22.3)</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>78.5 (11.8)</td>
<td>79.2 (11.6)</td>
</tr>
<tr>
<td>Peripheral arterial disease, %‡</td>
<td>16.9</td>
<td>16.3</td>
</tr>
<tr>
<td>Diabetes mellitus, %</td>
<td>7.1</td>
<td>7.7</td>
</tr>
<tr>
<td>BMI, kg/m</td>
<td>26.6 (3.4)</td>
<td>26.5 (4.2)</td>
</tr>
</tbody>
</table>

Abbreviations are defined in text. *Values represent unadjusted means (SD) or percentages. Differences between groups were tested by a χ² test for categorical data and a Students’ t test for continuous variables.
† Adjusted for age, sex, study site, BMI, peripheral arterial disease, and diabetes mellitus.
‡Defined as an ankle-brachial index <0.9.

10% were without periventricular or subcortical WMLs, respectively, whereas 5% had no WMLs at all.

Independent of their apoE genotype, subjects with hypertension had a significantly higher volume of subcortical WMLs than did subjects without hypertension (adjusted mean difference, 1.0; 95% confidence interval [CI], 0.8 to 1.2). The same was found for periventricular WMLs (adjusted mean difference, 0.9; 95% CI, 0.7 to 1.1). Exclusion of apoE ε2 carriers did not consistently change the results.

apoE ε4 carriers (n = 261) had a higher mean subcortical WML volume compared with the reference group (n = 568; adjusted mean difference, 0.5; 95%, CI 0.2 to 0.8). This was not found for periventricular WMLs (adjusted mean difference, 0.1; 95% CI, −0.2 to 0.5).

Participants with both hypertension and at least 1 apoE ε4 allele had the highest subcortical WML volumes and the highest degree of periventricular WMLs (Table 2). We detected a statistically significant interaction between hypertension and the presence of at least 1 apoE ε4 allele for subcortical WMLs (interaction term apoE ε4 allele × hypertension, P = 0.016). This interaction was not found for periventricular WMLs.

### Table 2. Degree of WMLs* by apoE ε4 status, Stratified for Hypertension†

<table>
<thead>
<tr>
<th>Subcortical</th>
<th>Periventricular</th>
</tr>
</thead>
<tbody>
<tr>
<td>apoE ε3ε3</td>
<td>apoE ε4+</td>
</tr>
<tr>
<td>Hypertension absent</td>
<td>0.91 (2.8)</td>
</tr>
<tr>
<td>Hypertension present</td>
<td>1.28 (2.7)</td>
</tr>
</tbody>
</table>

Abbreviations are as defined in text.
*Values represent mean values (SD) for the severity of subcortical (milliliter) and periventricular (degree) WMLs, respectively.
†Adjusted for age, sex, study site, BMI, peripheral arterial disease, and diabetes mellitus.
‡P = 0.016 for the cross product (interaction term) hypertension × apoE ε4.

In this marginally perfused periventricular area, WMLs may readily emerge, especially in the presence of chronic hypertension, whereas other vascular risk factors also predominantly seem to affect the periventricular white matter in contrast to the subcortical white matter. The joint effect of vascular risk factors and specific local anatomy in the periventricular white matter may be so predominant that the presence of an apoE ε4 allele is not necessary for the emergence of WMLs. However, the subcortical white matter may be more resistant against the influence of vascular risk factors, and it may be that only the combination of a disease-modifying factor, eg, an apoE ε4 allele and a vascular risk factor, is a sufficient cause for the emergence of WMLs in the subcortical area.
The explanation for the genetic factor is incompletely understood but is in keeping with the notion that the apoE ε4 allele is associated with an impaired response to cerebral damage. Neuronal repair, including dendrite formation and synaptogenesis, is an important process in restoring the integrity of the brain in response to injury, eg, after a period of ischemia. It is known that longstanding hypertension ultimately leads to ischemia in areas with an already marginal blood supply under physiologic conditions, such as the periventricular and subcortical white matter, eventually resulting in WMLs. Pathologically these areas are characterized by tissue injury with neuronal loss, arteriolosclerosis, and membrane damage such as demyelination. It is therefore plausible that the brain injury caused by hypertension in apoE ε4 carriers is more severe than in others. If the brain does not have the correct proteins to repair itself after lifelong vascular risk factor exposure, subsequent deterioration of brain function is to be expected, reflected in cognitive impairment or dementia. Indeed, a recent study confirmed that apoE ε4 carriers with hypertension are most severely affected with respect to cognitive impairment. Previous large studies, both in demented patients and in participants from the general population, failed to show an association between the apoE ε4 allele and WMLs. An explanation for the difference with previous studies may be that those investigated the relation between the apoE ε4 allele and WMLs, irrespective of the presence of concomitant vascular disease. There is gradually increasing evidence that it is the interaction between the apoE ε4 allele and vascular risk factors that results in vascular disease. WML volume may be up to 3 times higher in individuals with an apoE ε4 allele and unspecified vascular disease than in those with an apoE ε4 allele alone. However, in that study, it remained to be elucidated which vascular disease or risk factor was responsible for this interaction. In our study, we studied a well-defined vascular risk factor in detail and found that an interaction between hypertension and the apoE ε4 allele resulted in a significantly higher WML volume than in individuals with hypertension or the apoE ε4 allele alone.

In conclusion, our results suggest that apoE ε4 carriers are at increased risk for WMLs if they suffer from hypertension as well. Intervention studies are needed to investigate whether the interaction of hypertension with the apoE ε4 allele indeed is a causal link in the development of WMLs and the attendant cognitive impairment.

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References
 Editorial Comment

Not All Hypertensive Subjects Have Similar Risks for White Matter Lesions: Influence of Genetic Factors

White matter lesions (WML) are frequently found on magnetic resonance imaging (MRI) scans in stroke patients, in patients with cognitive decline or dementia, and in healthy—usually elderly—subjects who have vascular risk factors, especially arterial hypertension. However, many neurologists in their clinical practice have seen elderly subjects with a long history of improperly treated arterial hypertension who have only few WML. They have met also middle-aged subjects, with less severe, more recent, and properly treated arterial hypertension with already extensive WML. These clinical findings suggest that not all hypertensive subjects have similar risks of WML.

Genetic factors may explain differences in the susceptibility of the cerebral white matter to arterial hypertension: (1) cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) is clearly identified as a genetic disease due to a mutation in the Notch 3 gene, in which severe WML occur at an early stage of the disease in young subjects, even in the absence of vascular risk factors; (2) other genetic disorders may also lead to severe WML in the absence of vascular factors, eg, CARASIL (the R indicating recessive) and other nonNotch type angiopathies; familial amyloid angiopathies, hereditary endotheliopathy with retinopathy nephropathy and stroke, and cerebroretinal angiopathies; and (3) a greater correlation between the volumes of WML is observed between monozygotic twins than between dizygotic twins.

Among the various genes that potentially predispose to WML in the presence of arterial hypertension, the apolipoprotein E (APOE) gene is one of the best candidates: (1) the APOE which is encoded by the APOE gene plays a crucial role in lipid metabolism and neuronal repair after injury of any type; (2) the e4 allele of the APOE gene is associated with the presence of vascular risk factors, vascular events, and cognitive impairment; (3) the e4 allele of the APOE gene modulates the severity of Aβ amyloid deposits in animal models and in humans, especially in patients with white matter changes; and (4) e4 homozygotes exhibit more extensive WML than other genotypes.

In the current issue of Stroke, De Leeuw and coworkers present their investigation of the interactions between the APOE genotype and blood pressure levels in the pathogenesis of subcortical and periventricular WML in 971 subjects participating in the Rotterdam community-based study. They found that APOE e4 carriers have significantly more subcortical WML than APOE e3e3 carriers, irrespective of their level of blood pressure. Subjects with arterial hypertension and at least one APOE e4 allele had the highest amount of WML, but the interaction was significant only for subcortical WML. They conclude that the coexistence of an e4 allele and arterial hypertension is strongly associated with the presence of subcortical WML, while neither hypertension alone nor the presence of an e4 allele alone are. This interaction may reflect a decreased capacity for neuronal repair in the presence of 1 or 2 e4 alleles.

The results of this study support the hypothesis that the effect of arterial hypertension on the cerebral white matter is enhanced in e4 carriers. It may be a clue for explaining why middle-aged subjects with arterial hypertension are more likely to develop Alzheimer’s disease 15 years later: those hypertensive middle-aged subjects with e4 allele might develop more extensive WML, independently from the development of Alzheimer lesions, leading to an anticipation of the clinical onset of dementia because of the summation of Alzheimer lesions and WML.

This study opens a door for a selective prevention in high-risk subjects: lowering blood pressure might be more beneficial in e4 carriers, and an effective prevention of the development of WML might be beneficial in terms of prevention of cognitive decline, dementia, and stroke. These data are of major importance for understanding the interaction between arterial hypertension and WML, but they cannot influence our practice as a result yet without raising ethical questions. With the presence of at least one e4 allele being associated with a higher risk of Alzheimer’s disease, is it reasonable to look for this marker of increased risk in the presence of arterial hypertension unless an effective pathogenic treatment of the disease is available? For the moment, the best we can do is to treat all treatable vascular risk factors, to decrease the risks of vascular events, including stroke, and of cognitive impairment, including Alzheimer's disease associated with vascular lesions. The result provided by the Rotterdam study, however, leads to exciting perspectives for a more selective prevention in those patients who are the most likely to have cerebral consequences of arterial hypertension. Other genetic predisposing factors that can make hypertensive subjects prone to develop WML should also be studied and will raise less ethical issues: the D allele of the angiotensin-converting enzyme gene may be one of them because of its association with lacunar stroke, a subtype of ischemic stroke sharing the same underlying small-vessel disease than WML.

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References


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