MRI Cerebral White Matter Lesions and Paraoxonase

PON1 Polymorphisms

Three-Year Follow-Up of the Austrian Stroke Prevention Study

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Abstract—White matter lesions (WMLs) on magnetic resonance imaging (MRI) scans of older persons are thought to be caused by cerebral small-vessel disease. As they progress, these brain abnormalities frequently result in cognitive decline and gait disturbances, and their predictors are incompletely understood. Genetic risk factors have been implicated but remain undetermined so far. We examined whether 2 common polymorphisms of the paraoxonase (PON1) gene leading to a methionine (M allele)–leucine (L allele) interchange at position 54 and an arginine (B allele)–glutamine (A allele) interchange at position 191 are associated with the presence and progression of WMLs. We studied 264 community-dwelling subjects without neuropsychiatric disease (ages 44 to 75 years). All underwent vascular risk factor assessment, brain MRI, and PON1 genotyping. MRI scanning was repeated after 3 years. The extent and number of WMLs were recorded by 3 independent readers. Progression of WMLs was assessed by direct scan comparison. The final rating relied on the majority judgment of the 3 readers. The LL, LM, and MM genotypes were noted in 111 (42.0%), 118 (44.7%), and 35 (13.3%) subjects, respectively; the AA, AB, and BB genotypes occurred in 146 (55.3%), 98 (37.1%), and 20 (7.8%) individuals, respectively. Carriers of the LL genotype showed a nonsignificant trend toward more extensive WMLs and more frequently demonstrated lesion progression over the 3-year observation period (P = 0.03). The polymorphism at position 191 had no effect. Logistic regression analysis yielded age (odds ratio, 1.08/y), diastolic blood pressure (odds ratio, 1.05/mm Hg), and LL paraoxonase genotype (odds ratio, 2.65) to be significant predictors of WML progression. These data suggest that the LL PON1 genotype at position 54 influences the extent and progression of WMLs in elderly subjects. (Arterioscler Thromb Vasc Biol. 2000;20:1811-1816.)

Key Words: white matter lesions ■ cerebral small-vessel disease ■ paraoxonase ■ genetics

Magnetic resonance imaging (MRI) shows cerebral white-matter lesions (WMLs) in a large proportion of individuals above the age of 50 years.1 Histopathological studies have demonstrated that these changes occur in the presence of arteriolosclerosis and are correlated with widening of the perivascular spaces, perivascular demyelination, or lacunar infarcts.2 Such abnormalities may be recognized in otherwise-normal individuals but are likely to become associated with cognitive impairment and gait disturbances as they progress.1 Identification of individuals prone to the development and progression of WMLs is important, because early control of causal factors in high-risk groups could reduce their clinical consequences, which are a major source of disability in the elderly population. So far, it is unclear which factors other than advancing age1,3,4 and arterial hypertension5,6 predispose individuals to this type of ischemic brain damage. A significant contribution of genetic influence has only recently been demonstrated in a US study on World War II veteran twins.7 That investigation reported a probandwise concordance rate for severe WMLs of 61% in monozygotic twins and of 38% in dizygotic twins compared with a prevalence rate of 15% for the entire population. The estimated heritability of WML volume was 73%.

The strong association of WMLs with aging and a previous study of our own demonstrating an inverse relationship between lesion extent and plasma levels of antioxidants8 suggest that genes involved in oxidative defense could play a role in the etiology of such brain abnormalities. Free-radical formation increases significantly with aging,9 and increased lipid peroxidation and oxidative stress due to excess free-radical activity and impaired antioxidant defenses have been associated not only with large- but also with small-vessel disease.10,11 The most likely cause of WMLs in the elderly,2 Paraoxonase has antioxidative potential12 and could thus protect against both macrovascular and microvascular diseases, even though they represent distinct vascular pathologies. So far, there have been several publications relating paraoxonase to pathological phenotypes of large-vessel dis-
ease, such as coronary heart disease, and carotid atherosclerosis; a single study found an association with small-vessel disease–related diabetic retinopathy. The paraoxonase gene is located at q21 to q22 on the long arm of chromosome 7. The ability of paraoxonase to detoxify organophosphorus compounds has been known for years, and its enzyme activity had been determined earlier by the use of the pesticide paraoxon. White populations have a triphasic distribution of paraoxonase activity toward paraoxon, which is caused by an amino acid substitution at position 191. Glutamine (A allele) is replaced by arginine (B allele) in its high-activity isoform. The B allele has been shown to be associated with coronary heart disease. Another frequent polymorphism at position 54 leads to a methionine (M allele)–leucine (L allele) interchange. The 2 polymorphisms are in linkage disequilibrium with leucine at position 54, giving rise to arginine at position 191. We explored whether these genetic variants influence the occurrence and progression of WMLs in a large cohort of randomly selected, community-dwelling middle-aged and elderly individuals who have been followed up over a time period of 3 years.

Methods

Individuals and Study Design
The study population consisted of participants in the Austrian Stroke Prevention Study. The rationale and design of this study have been previously described. In brief, 1998 participants without a history or signs of neuropsychiatric disease were randomly selected from the official community register. They underwent 3 blood pressure readings, an ECG, and echocardiography, as well as laboratory testing including blood cell count and a complete blood chemistry panel. Every fourth, or in case of refusal, the next, study participant was invited to enter phase 2 of the study, which included MRI and Doppler sonography. From a total of 498 phase 2 participants, 458 volunteered to undergo an MRI study. At the second study panel, 3 years after baseline, we were able to contact 386 participants of the original MRI sample or their proxies. Seven subjects had died and 7 had experienced a stroke, which was an end point in our study. There were 27 subjects who did not want to undergo the extensive diagnostic work-up a second time. A total of 21 individuals had moved from the city, and 51 subjects could not be reached on the occasion of 3 phone calls and did not respond to a written invitation. The remaining 345 phase 2 participants agreed to be reexamined, but 72 individuals refused to have a second MRI scan because they had experienced claustrophobia and claustrophobic hyperventilation. Blood sampling for DNA extraction was done in all but 9 individuals. The current study cohort therefore comprises those 264 participants who underwent MRI scanning at baseline and at the 3-year follow-up and assessment of the PON1 polymorphisms. There were 128 women and 136 men. The mean ± SD age was 59.9 ± 6.1 years (median, 60.0 years) at baseline. The sample consisted exclusively of white subjects of central European origin; the length of education ranged from 9 to 18 years, with a mean of 11.7 years. The individuals who participated in the follow-up MRI study did not differ from those who dropped out in terms of age, sex, educational and occupational status, and risk factors for stroke. This 3-year follow-up of the Austrian Stroke Prevention Study is not prospective in design because no data on PON1 polymorphisms for 25% of the original group of subjects who volunteered to undergo a first MRI were available.

Vascular Risk Factors
Historical information and laboratory findings at baseline and follow-up were considered for diagnosis of arterial hypertension, diabetes mellitus, and cardiac disease, including embolicogenic abnormalities, coronary heart disease, and left ventricular hypertrophy. We also assessed smoking habits and body mass index (BMI). Lipid status including the levels of plasma triglycerides, total cholesterol, LDL cholesterol, HDL cholesterol, and Lp(a) lipoprotein, as well as measurement of plasma fibrinogen, was determined for each study participant at both examinations. A detailed description of the definitions of risk factors and of the laboratory methods used has been published previously. The means of systolic and diastolic blood pressures, fasting blood sugar, BMI, lipid, and fibrinogen values on baseline and follow-up measurements were calculated and used for data analyses.

Isolation of DNA and Genotype Analysis
High-molecular-weight DNA was extracted from peripheral whole blood by using Qiagen genomic tips (Qiagen Inc) according to the protocol supplied by the manufacturer. Genotyping of the Met-Leu54 polymorphism was done by polymerase chain reaction (PCR) amplification of a 170-bp-long fragment and using the primers described by Humbert et al. The PCR products were cleaved by NcoIII in the presence of BSA at 37°C for 3 hours. The digested products were analyzed on a 15% polyacrylamide gel, stained with ethidium bromide, and examined under UV transillumination. The L allele corresponded to the nondigested 170-bp-long fragment, whereas the M allele corresponded to 126- and 44-bp fragments. A similar protocol was used for genotyping the Gin-Arg191 polymorphism and also using the primers described by Humbert et al.

Magnetic Resonance Imaging
MRI was performed on 1.5-T superconducting magnets (Gyrosan S 15 and ACS. Philips) with proton-density and T2-weighted (repetition time [TR]/echo time [TE], 2000–2500/30–90 ms) sequences in the transverse orientation. T1-weighted images (TR/TE, 600/30 ms) were generated in the sagittal plane. Slice thickness was 5 mm and the matrix size used was 128×256 pixels. The MRI protocols at baseline and at the 3-year follow-up were identical. The scanning plane was always determined by a sagittal and coronal pilot to ensure consistency in image angulation throughout the study. The baseline and follow-up scans of each study participant were read independently by 3 experienced investigators who were blinded to the clinical data of study participants. Blinding of the readers for the date of the examinations was impossible because the format of hard copies had changed from baseline to follow-up. According to our scheme, WMLs included abnormalities in the subcortical region and deep white matter as well as irregular periventricular lesions extending into the deep white matter. They were graded into absent (grade 0), punctate (grade 1), early confluent (grade 2), and confluent (grade 3) abnormalities. The number of WMLs was recorded and categorized into 0, 1 to 4, 5 to 9, and >9 lesions. k Values for interrater agreement regarding WML grade at baseline and at 3 years ranged from 0.63 to 0.70 and from 0.60 to 0.68, respectively, in regard to the number of lesions. We disregarded caps and pencil-thin periventricular linings because they represent normal anatomic variants. A change of WMLs in grade or number from baseline was determined by direct scan comparison. The final rating of WML progression relied on majority judgment of the 3 assessors. In case of complete disagreement, consensus was found in a joint reading session. The interrater agreement for WML progression ranged from 0.59 to 0.68. The Figure displays examples for each WML grade and for WML progression. We also recorded lacunes. They were defined as focal lesions isointense to cerebrospinal fluid and involving the basal ganglia, the internal capsule, the thalamus, or the brain stem and not exceeding a diameter of 10 mm.

Carotid Artery Duplex Scanning
Color-coded equipment (Diasonic, Vingmed CFM 750) was used to determine vessel wall abnormalities of the carotid arteries in all participants. The imaging protocol, grading of atherosclerotic changes, and the associations between duplex findings and paraoxonase genotypes in our study population have been reported previously. In the current study, we describe the presence of atherosclerotic changes among the genotype subsets and adjust for this variable when assessing the influence of the paraoxonase polymorphisms on WMLs.
Statistical Analysis
We used the Statistical Package for the Social Sciences (SPSS 8.0) for data analysis. Categorical variables among the paraoxonase genotypes were compared by the \( \chi^2 \) test. Assumptions of a normal distribution for continuous variables were compared by Lilliefors statistics. Normally distributed continuous variables were compared by 1-way ANOVA, whereas the Kruskal-Wallis test was used for comparison of nonnormally distributed variables. Allele frequencies were calculated by the gene counting method, and Hardy-Weinberg equilibrium was assessed by the \( \chi^2 \) test. The relative contribution of the paraoxonase genotypes to the presence of WMLs at baseline and to WML progression at the 3-year follow-up was assessed by multiple logistic regression analysis. Forward-selection stepwise regression analysis was used to create a model of independent predictors of MRI findings. At each step, each variable not yet in the model was assessed for its contribution to the model, with the most significant variable to be added. This process continued until no further variable made a significant (\( P<0.05 \)) contribution to the model. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated from the \( \beta \) coefficients and their SEs.

Results
The \( LL \), \( LM \), and \( MM \) genotypes were noted in 111 (42.0%), 118 (44.7%), and 35 (13.3%) individuals, respectively. The \( AA \), \( AB \), and \( BB \) genotypes occurred in 146 (55.3%), 98 (37.1%), and 20 (7.8%) individuals, respectively. These frequencies are similar to those reported in other European populations.\(^{18,23} \) The genotypes of both polymorphisms were in Hardy-Weinberg equilibrium. There existed a moderate association between the 2 polymorphisms, with arginine at position 191 being, with 1 exception, always concurrent with leucine at position 54.

Table 1 compares demographic variables and risk factors among the \( LL \), \( LM \), and \( MM \) genotype subsets. Individuals with the \( MM \) genotype had slightly higher fasting glucose levels than did those with the \( LM \) or \( LL \) genotype, and there was a nonsignificant trend for lower triglyceride levels in \( LM \) carriers. These between-group differences diminished further after correction for antidiabetic and lipid-lowering treatment. There existed no significant difference among the \( AA \), \( AB \), and \( BB \) genotype subsets when the demographic variables and risk factors listed in Table 1 were compared (data not shown). Duplex scanning showed atherosclerotic changes of the carotid arteries in 71 (64.0%) subjects with the \( LL \) genotype but in only 56 (47.5%) \( LM \) and 18 (51.4%) \( MM \) carriers (\( P=0.04 \)). Carotid artery abnormalities were also seen in 81 (55.5%) \( AA \), 51 (52.0%) \( AB \), and 13 (65.0%) \( BB \) carriers (\( P=0.56 \)).

At baseline, 171 (64.8%) participants had WMLs on MRI. After 3 years, progression of abnormalities had occurred in 47 (17.8%) subjects. Regression of abnormalities was never observed by the majority of readers. A breakdown of WML findings by paraoxonase genotypes is given in Table 2. As shown in the Table, the \( PON1 \) polymorphisms had no significant influence on baseline results. Yet subjects homozygous for the \( L \) allele at position 54 showed a trend toward higher grades of WMLs at the initial MRI examination than did their counterparts with either the \( LM \) or \( MM \) genotype. Progression of lesions over 3 years occurred at a significantly higher frequency in the \( LL \) genotype subset. There was a significant association between WML findings at baseline and the presence of carotid atherosclerosis (\( P=0.017 \)). The association was mainly due to a higher frequency of grade 2 or 3 WMLs in subjects with carotid changes than in those with a normal sonographic examination (5.9% versus 16.6%). Progression of WMLs was not related to the presence of carotid atherosclerosis. At baseline, lacunes were found in a total of 17 subjects and were always seen in the basal ganglionic/thalamic region. At the 3-year follow-up, new lacunes had evolved in 8 subjects. There was no significant difference in the distribution of subjects with lacunes at baseline and with evolving lacunes at follow-up among the genotypes, but the numbers in the comparative subsets were small.

Logistic regression analysis yielded an unadjusted OR of 2.38 (95% CI, 1.25 to 4.53; \( P=0.008 \)) for progression of WMLs in the \( LL \) genotype relative to the 2 other genotypes. The OR after adjustment for age and sex was 2.55 (95% CI, 1.32 to 4.96; \( P=0.005 \)). Evaluation of the effect of the
Gln-Arg191 polymorphism demonstrated that WML progression occurred more commonly in BB carriers, but these differences with respect to the AA and BB genotypes were nonsignificant. The Gln-Arg191 polymorphism did not modulate the effect of the LL genotype on WML progression because lesion progression was seen at almost identical frequency in 11 (23.9%) subjects in the LL/AA group and in 5 (26.3%) individuals in the LL/BB group. When we used forward stepwise regression analysis to create a model of predictors of WML progression, the LL genotype remained in this model in addition to age and diastolic blood pressure. Age entered the model first, the LL genotype second, and diastolic blood pressure third (Table 3). All other variables, including sex, BMI, systolic blood pressure, fasting glucose level, diabetes, smoking status, cardiac disease, blood lipids, plasma fibrinogen level, and carotid atherosclerosis did not enter the model. When carotid atherosclerosis was forced into the model, the OR for the association between the LL genotype and WML progression did not materially change.

**Discussion**

We found that homozygosity for the L allele at position 54 tended to be associated with the extent of WMLs at baseline and predicted WML progression in addition to advancing age and diastolic blood pressure. As in the Cardiovascular Health Study, we have seen an association between WMLs and

**TABLE 1.** Demographics and Risk Factors Among Paraoxonase Leu-Met54 Genotypes

<table>
<thead>
<tr>
<th>Variable</th>
<th>LL (n=111)</th>
<th>LM (n=118)</th>
<th>MM (n=35)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>59.6±5.9</td>
<td>60.1±6.4</td>
<td>60.4±5.6</td>
<td>0.73*</td>
</tr>
<tr>
<td>Sex, male, n (%)</td>
<td>55 (49.5)</td>
<td>61 (51.7)</td>
<td>20 (57.1)</td>
<td>0.74†</td>
</tr>
<tr>
<td>Hypertension, n (%)</td>
<td>46 (41.4)</td>
<td>39 (33.1)</td>
<td>17 (48.6)</td>
<td>0.19†</td>
</tr>
<tr>
<td>Diabetes, n (%)</td>
<td>7 (6.3)</td>
<td>5 (4.2)</td>
<td>3 (8.6)</td>
<td>0.58†</td>
</tr>
<tr>
<td>Fasting glucose, mg/dL</td>
<td>95.3±20.9</td>
<td>92.4±14.2</td>
<td>101.8±30.1</td>
<td>0.07*</td>
</tr>
<tr>
<td>Cardiac disease, n (%)</td>
<td>50 (45.0)</td>
<td>47 (39.8)</td>
<td>13 (37.1)</td>
<td>0.61†</td>
</tr>
</tbody>
</table>

**TABLE 2.** Paraoxonase Genotypes and MRI WMLs: Baseline Findings and 3-Year Progression

<table>
<thead>
<tr>
<th>Variable</th>
<th>LL (n=111)</th>
<th>LM (n=118)</th>
<th>MM (n=35)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>WML grade, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>38 (34.2)</td>
<td>44 (37.3)</td>
<td>11 (31.4)</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>52 (46.8)</td>
<td>66 (55.9)</td>
<td>22 (62.9)</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>16 (14.4)</td>
<td>6 (5.1)</td>
<td>2 (5.7)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>5 (4.5)</td>
<td>2 (1.7)</td>
<td>0</td>
<td>0.08</td>
</tr>
<tr>
<td>WML number, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>38 (34.2)</td>
<td>44 (37.3)</td>
<td>11 (31.4)</td>
<td></td>
</tr>
<tr>
<td>1–4</td>
<td>31 (27.9)</td>
<td>41 (34.7)</td>
<td>14 (40.0)</td>
<td></td>
</tr>
<tr>
<td>5–9</td>
<td>22 (19.8)</td>
<td>16 (13.6)</td>
<td>5 (14.3)</td>
<td></td>
</tr>
<tr>
<td>&gt;9</td>
<td>20 (18.0)</td>
<td>17 (14.4)</td>
<td>5 (14.3)</td>
<td>0.70</td>
</tr>
<tr>
<td>Progression, n (%)</td>
<td>83 (74.8)</td>
<td>104 (88.1)</td>
<td>30 (85.7)</td>
<td>0.03</td>
</tr>
<tr>
<td>Absent</td>
<td>28 (25.2)</td>
<td>14 (11.9)</td>
<td>5 (14.3)</td>
<td>0.29</td>
</tr>
</tbody>
</table>

*χ² test.
carotid atherosclerosis at baseline, with carotid atherosclerosis being common among LL carriers. Yet the relationship between the Leu-Met54 polymorphism and WML progression occurred independently of extracranial carotid disease. We failed to detect a significant association between the Gln-Arg191 polymorphism and WML progression, although progression was more common in BB than in AB and AA carriers. The frequency of lesion progression in subjects with the combination of the LL/BB genotypes was virtually identical to that in the study participants with the combined LL/AA genotypes. This indicates that the Gln-Arg191 polymorphism has no effect on the natural course of WMLs per se and does not modulate the effect of the L allele.

In line with these results, Garin et al25 found that the Leu-Met54 polymorphism is of central importance to paraoxonase function because it influences the serum activity and concentration of the enzyme, whereas the 191 variant has only little effect. This finding, together with the inconsistent results of other studies on the association between the Gln-Arg 191 polymorphism and coronary heart disease in genetically distinct populations,26 suggests that the PON1 polymorphism at position 191 is not causal but rather may be in linkage disequilibrium with a functional sequence variant in the vicinity. Whether the Leu-Met54 polymorphism represents this variant cannot be elucidated in allelic association studies. PON1 belongs to a multigene family including PON2 and PON3 at the same locus on chromosome 7.27 Two polymorphisms in the PON2 gene have only recently been described. Their functional importance is not yet fully determined, but like the PON1 polymorphisms, they were linked to coronary heart disease.28

Several studies support the role of paraoxonase in atherogenesis. The enzyme is linked to HDL and may be partly responsible for the antioxidative effect of this lipid fraction.12,19 Paraoxonase decreases lipoperoxide accumulation on LDL,20 a process that takes place in the subendothelial space.20 In line with this presumed location of action, paraoxonase was found to be present in interstitial fluid in an enzymatically active form.21 Results in PON1 knockout mice have demonstrated that HDL from PON1-deficient mice is unable to prevent LDL oxidation in a coculture model of the arterial wall.32 Paraoxonase immunoreactivity is seen in atherosclerotic lesions, and its intensity increases with their progression.33 There exists much less information on the association between paraoxonase and microvascular disease, which is the most likely cause of WMLs.3 Yet a study of Kao et al17 investigated the role of PON1 polymorphisms in small-vessel disease–related diabetic retinopathy and found that the LL genotype at position 54 was associated with this condition, whereas there existed no effect of the Gln-Arg191 polymorphism. These results are consistent with our findings on WMLs. Importantly, in the context of our study results, Primo-Parmo et al27 reported PON1 expression in adult mouse brain and described sequence homologues isolated from a postnatal human brain cDNA library.

Conceivably, the functional significance of the PON1 polymorphism at position 54 is due to its effect on enzyme activity and concentration caused by altered gene expression.34 Unfortunately, we have no frozen serum from our study participants and therefore could not measure these variables in our study group.

In summary, our data suggest a moderate modulatory effect of the Leu-Met54 polymorphism of the PON1 gene on the extent and progression of small-vessel disease–related MRI WMLs. Identification of individuals at risk for an unfavorable evolution of these brain changes is important, because increasing lesion load commonly results in cognitive decline and other neurological signs and symptoms such as gait disturbances and a tendency to falls.1,35

**Acknowledgment**

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**References**


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