Paraoxonase PON1 Polymorphism Leu-Met54 Is Associated With Carotid Atherosclerosis

Results of the Austrian Stroke Prevention Study

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Background and Purpose—Genetic polymorphism at the paraoxonase locus is associated with serum concentration and activity of paraoxonase and with increased risk for coronary heart disease. Two frequent polymorphisms present at the paraoxonase gene are the methionine (M allele) leucine (L allele) interchange at position 54 and the arginine (B allele) glutamine (A allele) interchange at position 191. This is the first study to determine the effect of these polymorphisms on carotid atherosclerosis.

Methods—The paraoxonase genotypes at positions 54 and 191 of 316 randomly selected individuals aged 44 to 75 years were determined by polymerase chain reaction–based restriction enzyme digestion. Carotid atherosclerosis was assessed by color-coded Duplex scanning and was graded on a 5-point scale ranging from 0 (normal) to 5 (complete luminal obstruction).

Results—The LL, LM, and MM genotypes at position 54 were noted in 137 (43.4%), 132 (41.8%), and 47 (14.9%) subjects; the AA, AB, and BB genotypes at position 191 occurred in 172 (54.4%), 124 (39.2%), and 20 (6.3%) individuals. The LL genotype was significantly associated with the presence and severity of carotid disease ($P=0.022$), whereas the 191 polymorphism had no effect. Logistic regression analysis with age and sex forced into the model demonstrated plasma fibrinogen (odds ratio [OR], 1.005 per mg/dL), LDL cholesterol (OR, 1.01 per mg/dL), cardiac disease (OR, 1.75), and the paraoxonase LL genotype to be significant predictors of carotid atherosclerosis. The ORs for the associations with age and sex were 1.09 ($P=0.0003$) and 1.66 ($P=0.052$) per year.

Conclusions—These data suggest that the paraoxonase LL genotype may represent a genetic risk factor for carotid atherosclerosis. (Stroke. 1998;29:2043-2048.)

Key Words: atherosclerosis ▪ carotid arteries ▪ genetics ▪ paraoxonase

Carotid atherosclerosis is considered to be a major cause of ischemic stroke.1 In recent years, oxidative stress has been demonstrated to play an important role in the pathogenesis of atherosclerosis.2 Low-density lipoprotein (LDL) seems to be the major target of oxidative modification, making it particularly atherogenic.3,4 Identification of factors protecting against oxidative modification of LDL are therefore of major interest. High-density lipoprotein (HDL) has been shown to have antioxidative potential; however, the mechanism(s) of its action is not known.5 One mechanism might be the enzymatic removal of lipid peroxides accumulating on the LDL particle by enzymes present on HDL.6 Paraoxonase is tightly associated with HDL and has been shown to reduce the accumulation of lipid oxidation products on LDL.7,8

The human serum paraoxonase is a 43- to 45-kDa protein. Its gene is located at q21 to q22 on the long arm of chromosome 7.7 The amino acid sequence of paraoxonase is highly conserved among animal species, suggesting an important metabolic role for this enzyme.10 The ability of paraoxonase to detoxify organophosphorous compounds has been known for years. Its activity was determined earlier by the use of paraoxon, a widely used pesticide. The physiological substrate of paraoxonase is yet unknown. Watson et al11 reported recently that an oxidized phospholipid may represent a potential candidate. White populations have a triphasic distribution of serum paraoxonase activity towards paraoxon but not to other substrates such as phenylacetate.7 This difference in enzyme activity is caused by an amino acid substitution at position 191. Glutamine (A allele) is replaced by arginine (B allele) in the high-activity isoform.9 The B allele has been shown to be associated with coronary heart disease.12-14 Another frequent polymorphism present at position 54 involves a methionine (M allele) leucine (L allele) interchange.9 The 2 polymorphisms are in linkage disequilibrium, with leucine at position 54 giving rise for arginine at position 191.5 The suspected role of paraoxonase in the
protection of LDL against oxidative modification, and the positive association found between paraoxonase genotypes and coronary heart disease, prompted us to investigate the effect of both 54 and 191 polymorphisms on carotid atherosclerosis in a normal middle-aged and elderly population.

**Subjects and Methods**

**Study Population**

Individuals aged 44 to 75 years and stratified by sex and 5-year age groups were randomly selected from the official register of residents of the city of Graz, Austria. They received a written invitation to participate in the Austrian Stroke Prevention Study (ASPS), a single-center prospective follow-up study in our community. The study has been approved by the Medical Ethics Committee of the Karl-Franzens University of Graz. Written informed consent was obtained from all study participants. The rationale and design of the ASPS have been previously described. 18 Briefly, the objective of the study is to examine the frequency of cerebrovascular risk factors and their effects on carotid atherosclerosis, as well as on cerebral morphology and function, in the normal elderly. The inclusion criteria for the study were no history of neuropsychiatric disease and a normal neurological examination. From a total of 8193 individuals invited between September 1991 and March 1994, a sample of 2794 subjects agreed to participate, with 1998 individuals fulfilling the inclusion criteria. All study participants underwent a structured clinical interview, a physical and neurological examination, 3 blood pressure readings, ECG, and echocardiography, as well as laboratory testing including blood cell count and a complete blood chemistry panel. Every fourth study participant was then invited to enter phase II of the ASPS, which included Doppler sonography, MRI, SPECT, and neuropsychological testing. Since 1993, we started to establish a gene bank in all phase II attendees. The present study cohort consists of those 316 individuals who underwent both carotid duplex scanning and assessment of the paraoxonase polymorphisms. There were 158 women and 158 men. The mean age of this cohort was 60.0±6.1 years.

**Vascular Risk Factors**

Diagnosis of vascular risk factors relied on the individuals’ histories and appropriate laboratory findings. Arterial hypertension was considered present if a subject had a history of arterial hypertension with repeated systolic/diastolic blood pressure readings >160/95 mm Hg or if the readings at examination exceeded this limit. Diabetes mellitus was coded present if a subject was treated for diabetes at the time of the examination or if the fasting blood glucose level at examination exceeded 140 mg/dL. Cardiac disease was assumed to be present if there was evidence of cardiac abnormalities known to be a source for cerebral embolism,19 evidence of coronary heart disease according to the Rose questionnaire17 or appropriate ECG findings18 (Minnesota codes I: 1 to 3; IV: 1 to 3; or V: 1 to 2), or if an individual presented with signs of left ventricular hypertrophy on echocardiogram or ECG (Minnesota codes III: 1; or IV: 1 to 3). Study participants were defined as smokers if they currently smoked >10 cigarettes a day. From current smokers and ex-smokers smoking duration in years. The data on the amount of tobacco were converted into grams of tobacco consumed during the lifetime using the following conversion factors: 1 cigarette=1 g, 1 cheroot=3 g, 1 cigar=5 g. For measurements of hematocrit, blood obtained from a large antecubital vein without stasis.

The body mass index was calculated as weight (kg)/height (m²). The regular use of estrogen replacement therapy was recorded among all female study participants.

A lipid status including the level of triglycerides, total cholesterol, LDL and HDL cholesterol, as well as lipoprotein(a) [Lp(a)], apolipoprotein (apo) B, and apoA-I was determined for each study participant. Thirty minutes after venipuncture, the coagulated blood samples were centrifuged at 1600g for 10 minutes, and the serum was transferred to plastic tubes and analyzed within 4 hours. Triglycerides and total cholesterol were enzymatically determined using commercially available kits (Uni-Kit III “Roche” and MA-Kit 100 “Roche,” Hoffman-La-Roche). HDL cholesterol was measured by the use of the TDx REA Cholesterol assay (Abbott). LDL cholesterol was calculated by the equation of Friedewald. The Lp(a) concentration was determined by the electroimmunodiffusion method using a reagent kit containing monospecific anti-Lp(a) antiserum and Rapidiphor M3 equipment (Immuno AG). The levels of apoB and apoA-I were assessed by an immunoturbidimetric method using polyclonal antibodies and a laser nephelometer (Behringwerke AG). The plasma fibrinogen concentration was measured according to the Clauss method19 using the prescription and reagents of Behringwerke AG.

**Isolation of DNA and Genotype Analysis**

High-molecular-weight DNA was extracted from peripheral whole blood using Qiagen genomic tips (Qiagen Inc) according to the protocol of the manufacturers.

Genotyping of the Leu-Met54 polymorphism was done by polymerase chain reaction (PCR) amplification of a 170-bp-long fragment using the primers described by Humbert et al.9 The PCR products are cleaved by NlaIII in the presence of BSA at 37°C for 3 hours. The digested products are 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, while the M allele corresponded to a 126-bp and a 44-bp fragment. A similar protocol was used for genotyping the Glu-Arg-191 polymorphism using the primers described by Humbert et al.9

**Carotid Duplex Scanning**

Color-coded equipment (DiaSonic, VingMed CFM 750) was used to determine atherosclerotic vessel wall abnormalities of the carotid arteries. All B-mode images. Data were transferred to a Macintosh personal computer for postprocessing and storage on optical disks. The imaging protocol involved scanning of both common and internal carotid arteries in multiple longitudinal and transverse planes and has been previously described.20,21 The examinations were done by one experienced physician. Image quality was assessed and graded into good (common and internal carotid arteries clearly visible and internal carotid arteries detectable over a distance of ≥2 cm), fair (common and internal carotid arteries sufficiently visible and internal carotid arteries detectable over a distance of 2 cm), and poor (common and internal carotid arteries insufficiently visible or internal carotid arteries detectable over a distance of <2 cm). The image quality was good in 308 (97%) and fair in 8 (3%) individuals. It was never poor. Measurements of maximal plaque diameter were done in longitudinal planes, and the extent of atherosclerosis was graded according to the most severe visible changes in the common and internal carotid arteries as 0=normal, 1=vessel wall thickening (>1 mm), 2=minimal plaque (<2 mm), 3=moderate plaque (2 to 3 mm), 4=severe plaque (>3 mm), and 5=lumen completely obstructed. Assessment of the intrarater reliability of this score was done in 50 randomly selected subjects and yielded a κ value of 0.83.

**Statistical Analysis**

We used the Statistical Package for Social Sciences (SPSS/PC+*) for data analysis. Categorical variables among the paraoxonase genotypes were compared by χ² test. Assumption of normal distribution for continuous variables was tested by Lilliefors statistics. Normally distributed continuous variables were compared by 1-way ANOVA, whereas the Kruskal-Wallis test was used for comparison of non-normally distributed variables. ANCOVA and logistic multivariate regression analysis were used to adjust for possible confounding in the comparison of risk factors among paraoxonase genotypes. Allele frequencies were calculated by the gene counting method, and Hardy-Weinberg equilibrium was assessed by χ² test. To test the differences in sonographic score among the 3 genotypes at both
polymorphic sites. Kruskal-Wallis 1-way ANOVA was used. To assess the relative contribution of the paraoxonase genotypes on the presence of carotid atherosclerosis, we used multiple logistic regression analysis. The sonographic score was dichotomized into normal (grade 0) or abnormal (grade 1 through 5). Vessel wall thickening (grade 1) was considered to be abnormal because it has been shown to represent an early stage of atherosclerosis and to be associated with an increased risk for future stroke.22,23 Forward selection stepwise regression analysis with age and sex forced into the model, and the most significant variable was added to the model. This process continued until no variable not in the model made a significant (P<0.05) contribution. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated from the β coefficients and their standard errors.

Results

The genotypes LL, LM, and MM were noted in 137 (43.4%), 132 (41.8%), and 47 (14.9%) study participants. The AA, AB, and BB genotypes occurred in 172 (54.4%), 124 (39.2%), and 20 (6.3%) study participants, respectively. The genotypes of both polymorphisms were in Hardy-Weinberg equilibrium. As shown in Table 1, there was a moderate association between the 2 polymorphisms, with arginine at position 191 being with 1 exception always concurrent with leucine at position 54.

Table 2 compares demographic variables and risk factors among the LL, LM, and MM genotype subsets. There was no significant difference between groups, with the exception of lower glucose levels and less frequent cardiac disease in those with the LM genotype. The significant difference in blood glucose level remained unchanged after adjustment for age, sex, and cardiac disease (P=0.03) but was no longer present after correction for use of antidiabetic treatment (P=0.26). Correction for use of lipid-lowering treatment did not materially change the results of comparisons of blood lipids between the 3 investigational subsets. The between-group difference for cardiac disease remained significant after adjustment for age, sex, and glucose level (P=0.04). There was no significant difference among the AA, AB, and BB genotype subsets when the demographic variables and risk factors listed in Table 2 were compared (data not shown).

Sonographic scores among the 3 genotypes for both polymorphisms are shown in Table 3. Overall, there were 63 (47.7%) subjects with the LM genotype and 23 (48.9%) subjects with the MM genotype, but 86 (62.7%) subjects with the LL genotype showed an abnormal sonographic score. Subjects homozygous for the L allele had higher grades of carotid abnormalities than subjects with either the LM or MM genotype (P=0.022). Logistic regression analysis yielded an unadjusted OR of 1.86 (95% CI, 1.18 to 2.94; P=0.007) for abnormal sonographic findings in the LL genotype relative to the other 2 genotypes. The OR after adjustment for age and

### Table 1. Distribution of Paraoxonase Genotypes Defined by Amino Acid Substitution at Positions 54 and 191

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>AA</th>
<th>AB</th>
<th>BB</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>LL</td>
<td>47</td>
<td>71</td>
<td>19</td>
<td>137</td>
</tr>
<tr>
<td>LM</td>
<td>81</td>
<td>50</td>
<td>1</td>
<td>132</td>
</tr>
<tr>
<td>MM</td>
<td>44</td>
<td>3</td>
<td>0</td>
<td>47</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>172</td>
<td>124</td>
<td>20</td>
<td>316</td>
</tr>
</tbody>
</table>

A indicates glutamine; B, arginine at position 191; L, leucine; and M, methionine at position 54.

### Table 2. Demographics and Risk Factors Among Paraoxonase Leu-Met54 Genotypes

<table>
<thead>
<tr>
<th>Variable</th>
<th>Genotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LL (n=137)</td>
</tr>
<tr>
<td>Age, y</td>
<td>59.8±5.9</td>
</tr>
<tr>
<td>Sex, % male</td>
<td>64 (46.7)</td>
</tr>
<tr>
<td>Hypertension, %</td>
<td>41 (29.9)</td>
</tr>
<tr>
<td>Diabetes mellitus, %</td>
<td>10 (7.3)</td>
</tr>
<tr>
<td>Fasting glucose, mg/dL</td>
<td>95.5±23.0</td>
</tr>
<tr>
<td>Cardiac disease, %</td>
<td>57 (41.6)</td>
</tr>
<tr>
<td>Smoking status, %</td>
<td>67 (48.9)</td>
</tr>
<tr>
<td>Never smoker</td>
<td>50 (36.5)</td>
</tr>
<tr>
<td>Current smoker</td>
<td>20 (14.6)</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>26.8±3.7</td>
</tr>
<tr>
<td>Hematocrit, %</td>
<td>36.5±14.5</td>
</tr>
<tr>
<td>Triglycerides, mg/dL</td>
<td>157.2±100.8</td>
</tr>
<tr>
<td>Cholesterol, mg/dL</td>
<td>228.4±39.1</td>
</tr>
<tr>
<td>LDL cholesterol, mg/dL</td>
<td>148.4±36.6</td>
</tr>
<tr>
<td>HDL cholesterol, mg/dL</td>
<td>49.0±16.0</td>
</tr>
<tr>
<td>Lipo protein(a), mg/dL</td>
<td>26.3±29.0</td>
</tr>
<tr>
<td>Fibrinogen, mg/dL</td>
<td>308.4±75.1</td>
</tr>
</tbody>
</table>

*Kruskal-Wallis test, †χ² test; ‡1-way ANOVA.
sex was 1.98 (95% CI, 1.23 to 3.20; \( P = 0.005 \)) and 1.88 (95% CI, 1.16 to 3.05; \( P = 0.01 \)) when adjusting for age, sex, fasting glucose level, and cardiac disease. Evaluation of the effect of the Gln-Arg-191 polymorphism on carotid atherosclerosis demonstrated that subjects with the BB genotype had the highest prevalence of carotid abnormalities. This difference between the genotypes did not reach statistical significance (\( P = 0.481 \)).

The Gln-Arg-191 polymorphism did not modulate the effect of the LL genotype on carotid disease because atherosclerotic changes were present in almost identical frequency in 74 (62.7%) subjects in the LL/AA group and in 12 (63.2%) individuals in the LL/BB group.

The relative contribution of the paraoxonase LL genotype to the presence of carotid atherosclerosis was determined by stepwise forward logistic regression analysis (Table 4). Age and sex was forced in the model. This analysis demonstrated the LL genotype to be significantly and independently associated with carotid atherosclerosis (\( P = 0.014 \)). Plasma fibrinogen entered first (OR, 1.005 per mg/dL), LDL cholesterol second (OR, 1.012 per mg/dL), the LL genotype third (OR, 1.907), and cardiac disease fourth (OR, 1.748). No other variables such as total cholesterol, HDL cholesterol, triglycerides, hypertension, diabetes mellitus, smoking, hematocrit, or body mass index were entered into the model. The ORs for the associations with age and sex were 1.09 (\( P = 0.0003 \)) and 1.66 (\( P = 0.05 \)) per year, respectively.

**Discussion**

Our data suggest that the paraoxonase LL genotype at position 54 is a significant and independent predictor of carotid atherosclerosis in a middle-aged and elderly population. Homozygosity for the L allele is associated with higher frequency and severity of carotid abnormalities, whereas heterozygosity for this allele results in no risk increase.

We failed to detect a significant association between the Gln-Arg-191 polymorphism and carotid disease, although atherosclerotic lesions were more common in BB than in AB or AA carriers. We found that individuals with the combination of the LL/BB genotypes had frequency of atherosclerosis virtually identical to that of those with combined LL/AA genotypes. This indicates that the Gln-Arg-191 polymorphism has no effect on carotid atherosclerosis per se and does not modulate the effect of the L allele on atherosclerosis. The Gln-Arg-191 polymorphism was defined as the molecular basis for the difference of paraoxonase activity observed against the artificial substrate, paraoxon.9 It was frequently reported to be associated with coronary heart disease.12–14 Blatter-Garin et al24 reported for the first time that in whites the Leu-Met54 polymorphism was significantly and independently of the polymorphism at position 191 associated with the concentration and activity of paraoxonase. These authors also found that the LL genotype predicted coronary heart disease.24 Similarly to our results, the effect was present in LL homozygotes only, indicating a recessive effect of the L allele. Sanghera et al25 investigated the effect of the Gln-Arg-191 polymorphism on coronary heart disease in the genetically distinct populations of Chinese and Asian Indians and found a race-specific association with coronary heart disease with the B allele only in Indian cohort. The inconsistency of associations in different populations strongly indicates that the polymorphism at position 191 is not causally related to atherosclerosis but is rather a marker for a functional sequence variant in its vicinity. Whether the Leu-Met54 polymorphism represents this functional variant is unclear and cannot be elucidated by association studies.

**TABLE 3. Paraoxonase Genotypes and Duplex Score**

<table>
<thead>
<tr>
<th>Duplex Score</th>
<th>Genotype Leu-Met54</th>
<th>Genotype Gln-191-Arg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LL (n=137)</td>
<td>LM (n=132)</td>
</tr>
<tr>
<td></td>
<td>AA (n=172)</td>
<td>AB (n=124)</td>
</tr>
<tr>
<td>0</td>
<td>51 (37.2%)</td>
<td>69 (52.3%)</td>
</tr>
<tr>
<td></td>
<td>80 (46.5%)</td>
<td>57 (46.0%)</td>
</tr>
<tr>
<td>1</td>
<td>5 (3.6%)</td>
<td>7 (5.3%)</td>
</tr>
<tr>
<td></td>
<td>6 (3.5%)</td>
<td>6 (4.8%)</td>
</tr>
<tr>
<td>2</td>
<td>52 (38.0%)</td>
<td>38 (28.8%)</td>
</tr>
<tr>
<td></td>
<td>58 (33.7%)</td>
<td>41 (33.1%)</td>
</tr>
<tr>
<td>3</td>
<td>22 (16.1%)</td>
<td>10 (7.6%)</td>
</tr>
<tr>
<td></td>
<td>21 (12.2%)</td>
<td>11 (6.8%)</td>
</tr>
<tr>
<td>4</td>
<td>7 (5.1%)</td>
<td>8 (6.1%)</td>
</tr>
<tr>
<td></td>
<td>7 (4.1%)</td>
<td>9 (7.3%)</td>
</tr>
</tbody>
</table>

*Kruskal-Wallis test.

**TABLE 4. Final Logistic Regression Analysis: Predictors of Carotid Atherosclerosis**

<table>
<thead>
<tr>
<th>Variable</th>
<th>( \beta )</th>
<th>SE</th>
<th>df</th>
<th>( P )</th>
<th>OR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y*</td>
<td>0.082</td>
<td>0.022</td>
<td>1</td>
<td>0.0003</td>
<td>1.086/y</td>
<td>1.04–1.135</td>
</tr>
<tr>
<td>Sex*</td>
<td>0.507</td>
<td>0.261</td>
<td>1</td>
<td>0.0519</td>
<td>1.660</td>
<td>0.996–2.768</td>
</tr>
<tr>
<td>Fibrinogen, mg/dL</td>
<td>0.005</td>
<td>0.002</td>
<td>1</td>
<td>0.0057</td>
<td>1.005/mg/dL</td>
<td>1.001–1.009</td>
</tr>
<tr>
<td>LDL cholesterol, mg/dL</td>
<td>0.012</td>
<td>0.004</td>
<td>1</td>
<td>0.0028</td>
<td>1.012/mg/dL</td>
<td>1.004–1.019</td>
</tr>
<tr>
<td>LL paraoxonase genotype</td>
<td>0.646</td>
<td>0.264</td>
<td>1</td>
<td>0.0144</td>
<td>1.907</td>
<td>1.137–3.198</td>
</tr>
<tr>
<td>Cardiac disease</td>
<td>0.558</td>
<td>0.275</td>
<td>1</td>
<td>0.0423</td>
<td>1.748</td>
<td>1.020–2.997</td>
</tr>
</tbody>
</table>

*Forced into the model.
Several recent publications support the role of paraoxonase in atherosclerosis. Paraoxonase is tightly associated with antiatherogenic HDL. According to recent studies, only certain subfractions of HDL are able to reduce the risk of atherosclerosis. Results from apoA-I and apoA-II transgenic (tg) mice underline this assumption.\textsuperscript{26–28} ApoA-II tg mice are prone to atherosclerosis while apoA-I tg mice were found to be protected against it, even though both had significantly increased HDL levels compared with control mice.\textsuperscript{29} HDL isolated from the apoA-I tg mice had been shown to protect against the accumulation of lipid peroxides on LDL, whereas HDL from apoA-II tg mice had no similar effect. The loss of ability of the apoA-II HDL to protect against LDL oxidation was associated with a decreased level of paraoxonase. Substitution of apoA-II HDL with paraoxonase restored its antioxidative ability.\textsuperscript{30} Paraoxonase is associated with a certain subfraction of HDL also containing apoA-I and apoJ.\textsuperscript{7} This HDL subfraction seems to play a central role in the antioxidative effect of HDL.\textsuperscript{30} Navab et al\textsuperscript{31} have found that in HepG2 cell culture, minimally oxidized LDL induces an increase in the apol/paraoxonase ratio due to altered transcriptional rates. They also reported an increase in the apoJ/paraoxonase ratio in different animal models of atherosclerosis on atherogenic diet, such as in mice prone to atherosclerosis, in apoE knockout mice, and in LDL receptor knockout mice. Interestingly, normolipidemic patients with coronary artery disease also had a significantly higher apoJ/paraoxonase ratio than normolipidemic controls.\textsuperscript{30}

Moreover, paraoxonase immunoreactivity was found in atherosclerotic lesions, and the intensity of immunoreactivity in the arterial walls increased with the progression of atherosclerosis.\textsuperscript{35} Recently, it was described by the same authors that paraoxonase is present in the interstitial fluid in an enzymatically active form.\textsuperscript{30} This is in line with the hypothesis that paraoxonase prevents the accumulation of lipid peroxides on LDL, a process that has to take place in the subendothelial space. If paraoxonase is involved in atherogenesis by its ability to prevent accumulation of lipid peroxides on LDL, and if the association between the L allele and carotid atherosclerosis is causal, then one would expect that the L isoform is less effective in preventing the oxidative modification of LDL than the M isoform. A recent report from Mackness et al\textsuperscript{36} supports this hypothesis. These authors investigated the antioxidative effect of HDL isolated from individuals carrying the AA, AB, or BB genotype on Cu\textsuperscript{2+} induced oxidation of LDL. They found that HDL from BB subjects completely lost its ability to prevent LDL oxidation within 6 hours, whereas HDL from AB or AA individuals still kept 23% and 40% of its original protective activity, respectively. Given the strong linkage disequilibrium between the B and the L allele, it is most likely that the L isoform has similar effects. In line with this suggestion, another recent work from Mackness et al\textsuperscript{37} showed that HDL from subjects with the MM/AA genotype most effectively protects oxidative modification, whereas HDL from subjects with the LL/BB genotype has the lowest antioxidative potential.

Several groups reported that the genotype at the paraoxonase locus influences the concentration and/or activity of serum paraoxonase, which partly maybe due to an altered expression of the paraoxonase gene.\textsuperscript{36} A weakness of our study is that due to the lack of frozen serum from our participants, we were not able to measure paraoxonase concentration and activity to test the effect of genotype on these parameters in our collective.

Our study is an allelic association study and cannot provide an explanation for the mechanism(s) of the paraoxonase genotypes leading to carotid disease. It might be that there exists a true causal relationship between leucine at position 54 in the paraoxonase enzyme and atherosclerosis; however, the possibility that the association is due to a linkage disequilibrium between the L allele and another functional allele in its neighborhood cannot be excluded. The PON1 gene is a member of a multigene family including PON2 and PON3 located at the same locus on chromosome 7.\textsuperscript{37} Recently, Hegele et al\textsuperscript{38} and Sanghera et al\textsuperscript{39} described 2 polymorphisms present in the PON2 gene with possible clinical relevance. However, function of the PON2 gene product is still unknown, making the estimation of the importance of these findings difficult.

In summary, this is the first report on a positive association between the paraoxonase LL genotype and carotid atherosclerosis. Independent of other vascular risk factors, homozygosity for the L allele was associated with a 1.91-fold increased risk for carotid disease. If our results can be confirmed in other ethnic groups, the Leu-Met54 variant, which can be easily determined by conventional DNA technology, may be considered to be included in the early risk assessment for stroke.

**Acknowledgments**

This project was partly supported by the Austrian Research Foundation Project P1691, SFB702 (Dr Kostner) and by the Franz Lanyar Stiftung of the Karl-Franz University (Dr H. Schmidt), Graz, Austria. The excellent technical assistance of Johann Semmler is appreciated.

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Stroke. 1998;29:2043-2048
doi: 10.1161/01.STR.29.10.2043
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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