Relationship Between High-Density Lipoprotein Paraoxonase Gene M/L55 Polymorphism and Carotid Atherosclerosis Differs in Smoking and Nonsmoking Men

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The enzyme paraoxonase (PON) can eliminate lipid peroxides and is believed to protect against low-density lipoprotein oxidation. A common polymorphism in the PON gene (*PON1*) causes an amino acid substitution of methionine (M) for leucine (L) at position 55 in the protein, which changes the activity of PON and can affect the risk of atherosclerosis. Because smoking is associated with increased lipid peroxidation, we studied the relationship between PON M/L55 polymorphism and the carotid artery intima-media thickness (IMT) in smokers or previous smokers (n = 112) and nonsmokers (n = 87). IMT was measured at 3 standardized segments by B-mode ultrasonography, and the overall mean IMT value of 199 randomly selected men (mean age 54.2 ± 3.0 years) was calculated. Subjects with IMT > 1.7 mm in at least 1 standard site were considered to have carotid artery atherosclerotic disease (CAAD). For analysis, L55 homozygotes were compared with the M55 allele carriers. Nonsmoking L55 homozygotes had an 8.9% (95% confidence interval [CI], 1.6 to 16.8) higher overall mean IMT than M55 allele carriers. In smokers, however, the M55 allele carriers tended to have higher overall mean IMT values than L55 homozygotes. There was also a statistically significant interaction between M/L55 genotype and smoking status on CAAD (*P* = .009) by logistic regression analysis. Among nonsmokers, the L55 homozygotes had an odds ratio of 4.22 (95% CI, 1.06 to 16.8) for CAAD compared with nonsmoking M55 allele carriers. Contrary to nonsmokers, the smoking M55 allele carriers had an odds ratio of 2.22 (95% CI, 0.82 to 6.01) for CAAD when the L55/L55 genotype of smokers was a reference group. These data suggest that in nonsmoking men, a PON L55/L55 genotype may represent a genetic risk factor for CAAD. The reverse effect in smokers implies that the ability of PON to protect against CAAD is influenced by cigarette smoking. The efficiency of this inhibition probably depends on the PON M/L55 genotype.

ATHEROSCLEROTIC cardiovascular diseases are one of the most common causes of morbidity and mortality in developed societies.[1] Oxidized low-density lipoprotein (LDL) cholesterol plays an important role in the pathogenesis of atherosclerosis.[2,3] Among other effects, cigarette smoking causes lipid peroxidation,[4,5] and it has been suggested that lipid peroxidation contributes to the increased risk of premature atherosclerosis and coronary heart disease (CHD) among smokers.[6] Metabolic activity of high-density lipoprotein (HDL) can prevent the oxidation of LDL, which might be mediated by certain enzymes.[7] Paraoxonase (PON) is one of the enzymes located on HDL particle, and it has been shown *in vitro* that PON reduces the accumulation of lipid peroxidation products on LDL.[8] Furthermore, it has been demonstrated that PON hydrolyzes lipid peroxides also in human atherosclerotic lesions,[9] strengthening the hypothesis that PON is also antiatherogenic *in vivo*.

Activity of PON toward an artificial substrate, paraoxon, is genetically determined by 2 common coding region polymorphisms in the *PON1* gene.[10] The first polymorphism leads to the substitution of arginine (R) for glutamine (Q) at position 192 (R/Q192).[11] Reports of reduced PON levels in patients after myocardial infarction[12,13] encouraged the search for an association between the R/Q192 polymorphism and CHD. Since then, several studies have shown a positive association between PON R192 allele and coronary disease,[14-19] and several other studies, including 2 studies from Northern Europe, have shown no association.[20-25] The second polymorphism of the *PON1* gene leads to the substitution of methionine (M) for leucine (L) at position 55 (M/L55).[26] The M/L55 polymorphism modulates a component of enzyme's activity against paraoxon and possibly also against some naturally occurring substrate(s).[8] The higher activity against paraoxon is assigned to the L55 variant and lower activity to the M55 variant.[8] The M/L55 polymorphism is also one of the determinants of serum concentration of the enzyme.[8,10] Few studies have attempted to study the association between M/L55 polymorphism and CHD.[10,17,27-29] In some of these studies, the L55 allele was associated with the risk of CHD.[10,29]

High-resolution ultrasonography allows for noninvasive and quantitative assessment of atherosclerotic changes in the peripheral vascular wall.[30-32] Age, high systolic blood pressure, smoking, and high LDL cholesterol concentration are all directly proportional to the carotid artery intima-media thickness (IMT) measured by B-mode ultrasonography.[30,33] Further, carotid atherosclerosis is considered a major cause of ischemic stroke,[34] and a connection has been described between coronary artery disease and carotid artery atherosclerotic disease (CAAD).[35] To our knowledge, only one previous study has assessed the relationship between PON M/L55 polymorphism and CAAD.[29] However, that study did not specifically address the impact of smoking. We have investigated the association between CAAD and the PON M/L55 genotype as modulated by smoking. We used ultrasonography to measure the carotid IMT

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Submitted November 7, 2000; accepted February 2, 2001.

Supported by the grants from the Medical Research Fund of Tampere University Hospital, the Finnish Foundation for Cardiovascular Research, and the Finnish Ministry of Education.

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0026-0495/01/5009-0011$35.00/0

doi:10.1053/meta.2001.25641

and related the IMT to PON M/L55 genotypes according to smoking status, with the intention of assessing their role in CAAD.

SUBJECTS AND METHODS

Subjects

Subjects were randomly selected from a total cohort of 9,058 men aged 50 to 59 years living in the city of Tampere in southern Finland. Three hundred subjects were invited to participate the study, and 223 agreed to participate (74%); 33 refused, and 44 did not answer or could not be reached. All required data were obtained for 199 of these participants, and these data constituted the final analysis of clinical characteristics and carotid IMT. The study was approved by the ethics committee of the UKK Institute, and all participants gave written informed consent. Detailed medical histories were collected, with particular emphasis on cardiovascular and metabolic diseases, smoking habits, and chronic medication. Weight and height were recorded, and the body mass index (BMI) was calculated (kg/m^2). Blood pressure was recorded from the dominant arm by use of a mercury sphygmomanometer after the subject had rested supine for 15 minutes. Three blood pressure measurements were taken with the subject supine, 2 standing, and 1 sitting, and the average of these measurements was recorded as the resting systolic and diastolic blood pressure values. The subject was considered to have hypertension if he was taking antihypertensive medication or had a systolic blood pressure of >160 or diastolic blood pressure of >95 mm Hg. There were 112 smokers (including 40 current smokers and 72 former smokers) and 87 nonsmokers in the study population. None of the subjects had had a symptomatic cerebrovascular event. Thirty-one subjects were treated for hypertension, 10 for diabetes mellitus, 9 for CHD, 4 for hyperlipidemia, and 17 for other diseases.

Biochemical Determinations

Blood was drawn after the subjects had fasted 12 hours. Lipoprotein fractions were assessed from fresh samples after ultracentrifugation.[36] Cholesterol was measured from serum and lipoprotein fractions using an enzymatic method (CHOD-PAP; Boehringer Mannheim, Mannheim, Germany). Triglycerides were determined from frozen samples by enzymatic hydrolysis (GPO-PAP; Boehringer Mannheim) Apolipoprotein B (apoB) was analyzed by immunonephelometry (Behring, Behringwerke AG, Marburg, Germany) and lipoprotein (a) [Lp(a)] by 2-site immunoradiometry (Pharmacia, Uppsala, Sweden).

Paraoxonase Genotyping

DNA was isolated from lymphocytes by use of a commercial kit (Qiagen Inc, Valencia, CA). The PON M/L55 genotypes were determined by a polymerase chain reaction and restriction enzyme *Hsp92*II (Promega, Madison, WI) digestion as described earlier.[26] The amplification cycle was performed in a PTC-225 thermal cycler (MJ Research Inc, Watertown, MA). After initial denaturation at 96°C for 2.5 minutes, the DNA was amplified by 40 cycles of denaturation at 96°C for 30 seconds, annealing at 61°C for 1 minute, and extension at 72°C for 1 minute. *Hsp92*II enzyme digestion was performed under conditions recommended by the manufacturer. Digested fragments were separated by electrophoresis on 2.5% agarose gel and visualized with ultraviolet light after ethidium bromide staining.

Carotid Ultrasonography

Quantitative carotid artery ultrasonography was performed using a standardized protocol adapted to the Finnish population.[32,37] A high-resolution B-mode ultrasound device with a 10-MHz transducer was used (Biosound Phase 2, Indianapolis, IN) to examine the left and right carotid arteries. The examinations were recorded on S-VHS videotapes, and the tapes were then read off-line at the ultrasound reading center, Wake Forest University, Winston-Salem, NC. All recordings and measurements were performed by 1 certified sonographer and 1 reader, respectively.

The right and left carotid arteries from both sides were imaged by a circumferential scan including the longitudinal views of the lateral, posterior, and anterior angles. The arteries were identified by Doppler analysis. The protocol involved scanning of the distal 10 mm of the common carotid artery, the bifurcation, and up to 10 mm of the proximal internal carotid artery. The distance between the media-adventitia interface and the lumen-intima interface is the IMT. The maximum IMT of the near and far wall was measured at 12 well-defined arterial segments. The single largest IMT was determined by selecting the largest IMT among the individual maximum IMTs in the 12 standard arterial walls, ie, the near and far walls of the common carotid artery, bifurcation, and the internal carotid artery at both sides. The mean maximum IMT (MMax IMT, overall mean) was the mean of 12 maximum IMTs identified at 12 standard sites.[32] To assess the intraobserver variability of the recording and measurement of IMT, a repeated scan of a total of 15 randomly selected participants were performed 1 week later. The mean absolute difference between repeated measurements was 0.052 mm in the MMax IMT of the 12-site, and the single largest mean difference, 0.11 mm, was detected in the near wall of the left internal carotid artery. The reproducibility of the measurements was good (eg, coefficient of variation [CV], 4.1% to 8.6% for common carotids), and these figures are comparable to previously reported data.[38] CAAD was defined as IMT > 1.7 mm in at least 1 site. The cut-off limit (1.7 mm) was calculated as overall mean IMT + 2 SD. The prevalence of CAAD was 21% when this cut-off limit was used.

Statistical Analysis

Calculations were done by the SPSS version 9.0 for Windows on a PC. Because the number of M55 homozygotes was small, these subjects were combined for analysis with the M55/L55 heterozygotes to get 2 groups: M55 allele carriers and L55 homozygotes. Non–normally distributed data such as triglycerides and Lp(a) concentrations and carotid IMTs were analyzed after logarithmical transformation, but the results are expressed as crude. The differences in mean IMTs between the 3 PON M/L55 genotypes and smoking status were analyzed by 2-way analysis of covariance (ANCOVA), in which the carotid IMT of the different segments was the dependent variable, the PON M/L55 genotype group and smoking subgroup were factors, and age, BMI, systolic blood pressure, LDL cholesterol, HDL cholesterol, apoB, and Lp(a) were the covariates. Discontinuous variables were compared using the χ^2 test. Logistic regression analysis was used to determine the adjusted odds ratios for CAAD according to PON M/L55 genotype groups. Data are expressed as means ± SD. A P value of <.05 was considered statistically significant.

RESULTS

The PON M/L55 genotype frequencies among the 199 men were 78 (39%) L55/L55, 90 (45%) M55/L55, and 31 (16%) M55/M55. The allele frequencies were 62% L55 and 38% M55. These figures are consistent with a previous study from Finland.[39] The genotypes were in Hardy-Weinberg equilibrium. Allele M55 carriers were combined into 1 group, and this group was compared with the L55 homozygotes. Table 1 shows the clinical characteristics of all 199 participants. There were no statistically significant differences in means with respect to traditional risk factors, eg, age, smoking status, BMI, and lipoprotein concentrations, or CAAD and hypertension status between the M55 allele carriers and L55 homozygotes. The

Table 1. Clinical Characteristics of the Study Population by Paraoxonase Genotype

	LL	M Allele Carriers	All
No. of subjects	78	121	199
Age (yr)	53.8 ± 2.8	54.4 ± 3.1	54.2 ± 3.0
BMI (kg/m^2)	27.3 ± 3.4	26.9 ± 3.7	27.0 ± 3.6
Systolic blood pressure (mm Hg)	128 ± 15	133 ± 17	131 ± 17
Diastolic blood pressure (mm Hg)	82.5 ± 9.9	84.6 ± 10.4	83.8 ± 10.2
Total cholesterol (mmol/L)	5.42 ± 0.92	5.47 ± 0.86	5.45 ± 0.89
LDL cholesterol (mmol/L)	3.53 ± 0.88	3.54 ± 0.77	3.53 ± 0.81
HDL cholesterol (mmol/L)	1.22 ± 0.32	1.23 ± 0.23	1.23 ± 0.27
VLDL cholesterol (mmol/L)	0.67 ± 0.44	0.70 ± 0.42	0.69 ± 0.43
Triglycerides (mmol/L)	1.49 ± 0.92	1.56 ± 0.83	1.53 ± 0.86
Lp(a) (mg/L)	239 ± 257	274 ± 384	260 ± 340
ApoB (g/L)	1.29 ± 0.32	1.30 ± 0.27	1.30 ± 0.29
CAAD status (yes/no)*	14/64	28/93	42/157
Hypertension (yes/no)*	10/68	21/100	31/168
Current smokers [n (%)]*	14 (17.9)	26 (21.5)	40 (20.1)
Former smokers [n (%)]*	29 (37.2)	43 (35.5)	72 (36.2)
Nonsmokers [n (%)]*	35 (44.9)	52 (43.0)	87 (43.7)
Pack-years of smoking (yr)	10.3 ± 12.0	12.4 ± 13.9	11.6 ± 13.2

NOTE. Values are means ± SD. There were no statistical significant differences between genotype groups by ANCOVA (age was used as covariate) or by χ^2 test*.

M55 allele carries and the L55 homozygotes also did not differ in the use of medication or alcohol. The pack-years of smoking were 30.7 ± 5.2 in current smokers and 16.3 ± 9.3 in former smokers. There were no differences in these numbers between the 2 PON M/L55 genotype groups.

Table 2 shows the mean IMTs in different segments of the carotid artery of nonsmokers and smokers and the mean percentual differences of these values by PON M/L55 genotype. There was a statistically significant association between the PON M/L55 genotype and the common carotid artery IMT in the group of nonsmokers when data were adjusted for covariates. The L55 homozygotes had 7.8% (95% CI, 1.7 to 14.2) higher mean IMT than the M55 allele carriers ($P = .012$). Among smokers, the common carotid artery IMT did not vary statistically significantly with PON M/L55 genotype in the ANCOVA model.

In nonsmokers, the L55 homozygotes tended to have higher mean IMT of the carotid bifurcation than the M55 allele carriers ($P = .056$). The interaction between M/L55 genotype and smoking status was statistically significant, and the genotype groups of smokers and nonsmokers behaved differently ($P = .009$): the smokers with the M55 allele had higher mean IMT, and the nonsmokers who carried the M55 allele had lower mean IMT than the L55 homozygotes.

The internal carotid artery did not vary statistically significantly among nonsmokers ($P = .073$) or smokers with PON M/L55 genotype in the ANCOVA model. However, the interaction between genotype and smoking status was statistically significant ($P = .031$).

In nonsmokers, the MMax carotid artery IMT varied significantly by PON M/L55 genotype subgroup in the ANCOVA. The L55 homozygotes had, on average, 8.9% (95% CI, 1.6 to

Table 2. Mean Carotid Artery IMT in Smokers and Nonsmokers in Different Segments of the Carotid Artery and Mean Percentual Differences of these IMTs by Paraoxonase Genotype

	LL (mean ± SD)	n	M Allele Carriers (mean ± SD)	n	LL *v* M Allele Carriers [% diff. (95% CI)]*†	PON–Smoking Interaction (*P*)†
Common carotid artery						
Smokers	1.04 ± 0.15	43	1.07 ± 0.23	69	2.1 (−3.7 to 8.2)	
Nonsmokers	1.06 ± 0.15	35	0.99 ± 0.17	52	7.8 (1.7 to 14.2)	.118
Bifurcation						
Smokers	1.38 ± 0.25	43	1.53 ± 0.41	69	−6.4 (−14.4 to 2.4)	
Nonsmokers	1.44 ± 0.35	35	1.31 ± 0.30	52	9.3 (−0.3 to 19.8)	.009
Internal carotid artery						
Smokers	1.09 ± 0.38	43	1.25 ± 0.57	69	−7.0 (−18.9 to 6.7)	
Nonsmokers	1.09 ± 0.33	35	0.98 ± 0.23	52	10.5 (−1.0 to 23.4)	.031
Overall mean IMT						
Smokers	1.18 ± 0.19	43	1.28 ± 0.29	69	−3.5 (−10.4 to 3.9)	
Nonsmokers	1.20 ± 0.22	35	1.10 ± 0.19	52	8.9 (1.6 to 16.8)	.009

*Values are mean percentual differences and their 95% confidence intervals between PON genotype groups.

†ANCOVA: age, BMI, systolic blood pressure, LDL cholesterol, HDL cholesterol, apoB, and Lp(a) were used as covariates. Data were transformed logarithmically before analysis.

16.8) higher MMax IMT values than the M55 allele carriers ($P = .017$). Among smokers, the association between MMax carotid artery IMT and genotype was not statistically significant. By 2-way ANCOVA, there was a statistically significant PON M/L55 genotype–smoking status interaction in the MMax carotid artery IMT ($P = .009$) adjusted for covariates when smokers and nonsmokers were compared. When the data were analyzed similarly but without combining the M55/L55 heterozygotes and M55 homozygotes, there was a lack of stepwise association between the 3 genotypes and mean IMT of different segments of carotid artery in both smokers and nonsmokers. However, in such analysis the group size of M55 homozygotes was only 15 in smokers and 16 in nonsmokers.

All subjects considered, smokers and former smokers had higher overall mean IMT than nonsmokers (1.22 ± 0.26 *v* 1.14 ± 0.21; $P = .038$, ANOVA). Among smokers and former smokers 31 (28%) of 112 subjects were classified as having carotid artery atherosclerosis, while only 11 (13%) of 87 nonsmokers had CAAD ($P = .010$, χ^2 test). Table 3 shows the odd ratios for CAAD among smokers and nonsmokers by PON M/L55 genotype groups according to logistic regression analysis. The interaction between M/L55 genotype and smoking status with regard to CAAD was statistically significant ($P = .009$) by logistic regression, in which age, BMI, systolic blood pressure, LDL cholesterol, HDL cholesterol, apoB, and Lp(a) were entered into the model as confounding variables. Nonsmoking L55 homozygotes had an odds ratio of 4.22 (95% CI, 1.06 to 16.8) for CAAD compared with nonsmoking M55 allele carriers. Contrary to nonsmokers, the odds ratio for CAAD was 2.22 (95% CI, 0.82 to 6.01) for the smoking M55 allele carriers compared with L55/L55 genotype smokers. When nonsmoking and smoking M55 allele carriers were compared, the smokers had an odds ratio of 6.45 (95% CI, 1.97 to 21.0) for CAAD.

In the logistic regression analysis, which was done without combining the M55/L55 and M55/M55 groups, the odds ratio for CAAD in nonsmokers increased linearly in the PON genotype order of M55/M55, M55/L55, and L55/L55. However, the increased odds ratio among smoking M55 allele carriers was caused by a higher prevalence of CAAD in smoking M55/L55 heterozygotes (22/54) than in M55 homozygotes (2/15).

DISCUSSION

Cigarette smoking increases the incidence of premature atherosclerosis and CHD.[6] This was also seen in our study, in which smokers and former smokers had more carotid atherosclerosis than nonsmokers. However, the reasons smoking is a risk factor for atherosclerosis are not clear. The results of the present study suggest that the PON M/L55 genotype may be involved. In this study, we examined specific sections of carotid arteries and related the IMTs to PON M/L55 genotypes in smokers and nonsmokers. We found a strong interaction between PON M/L55 genotype and smoking both by 2-way ANCOVA and by logistic regression analysis: The nonsmoking L55 homozygotes had higher MMax IMT than M55 allele carriers. Also, in the logistic regression analysis, the odds ratio for CAAD among nonsmokers was higher in L55 homozygotes than in M55 allele carriers, suggesting that the L55/L55 genotype might be a risk factor for carotid atherosclerosis in nonsmokers. In smokers, contrary to nonsmokers, the M55 allele carriers tended to have higher MMax IMT values than L55 homozygotes, and the same trend was observed in the segments of the carotid arteries. In the logistic regression model, the same unexpected phenomenon was seen. The prevalence of CAAD was higher among M55 allele carriers than among L55 homozygotes. This suggests that the ability of PON to protect against CAAD is influenced by cigarette smoke, and the efficiency of this inhibition probably depends on the PON M/L55 genotype. When the data were analyzed without combining the M55/L55 and M55/M55 groups, there was a lack of stepwise association between PON genotype and IMTs in both smokers and nonsmokers. Also, in the logistic regression analysis, the reason for a high odds ratio for CAAD among smoking M55 allele carriers was a high prevalence of CAAD in smoking M55/L55 heterozygotes rather than in M55 homozygotes. The loss of the stepwise associations may be attributable to small group size among M55 homozygotes, although it is possible that some unknown factors may be involved.

Minimal information is available at the molecular level concerning the mechanism of action of cigarette smoke. Nishio and Watanabe[40] recently demonstrated that a cigarette smoke extract inhibits PON activity in a dose- and time-dependent manner. Inhibition of PON by smoke might be caused by steric hindrance resulting from the introduction of a large substituent near a region of the molecule critical for substrate binding or the maintenance of an active enzyme conformation.[41] However, nothing is known about the effect of the PON genotypes on inhibition of PON activity caused by smoking. In our study, the effect of the PON M/L55 genotype on the development of CAAD was almost opposite among smokers and nonsmokers. This might mean that inhibition of PON activity by cigarette smoke varies according to PON M/L55 genotype. This hypothesis is supported by a recent study in which a statistically significant interaction between PON R/Q192 polymorphism and smoking status was found.[42] In that study, PON R192 allele

Table 3. Odds Ratios for Carotid Artery Atherosclerotic Disease Among Smokers and Nonsmokers by Paraoxonase Genotype

	Nonsmokers	Smokers
OR (95% CI) for LL	4.22 (1.06 to 16.8)	2.90 (0.74 to 11.4)
No. of subjects with CAAD (%)	7 (20)	7 (16)
OR (95% CI) for M allele carriers	1.00*	6.45 (1.97 to 21.0)
No. of subjects with CAAD (%)	4 (8)	24 (35)

NOTE. 95% CI adjusted for age, BMI, systolic blood pressure, LDL cholesterol, HDL cholesterol, apoB and Lp(a). $P = .009$ for PON–smoking interaction, $P = .042$ for PON genotype, $P = .544$ for smoking.

Abbreviation: OR, odds ratio.

*Reference category.

was associated with increased risk of myocardial infarction, but this association was evident only among nonsmokers. Smokers had an increased risk of infarction regardless of their PON R/Q192 genotype. However, the Q192 homozygotes who smoked tended to have higher odds ratios for infarction than R192 allele carriers. In another recent study, the risk of myocardial infarction associated with smoking was increased in subjects with PON Q192/Q192 genotype.[43] Our findings are in line with these observations because of linkage disequilibrium between the 2 PON polymorphisms, which favors the simultaneous presence of R at position 192 and L at position 55.[8,10] Several studies have been conducted to evaluate the possible relationship between PON genotypes and the risk of atherosclerotic diseases, but smokers and nonsmokers have not usually been analyzed separately. Based on the 2 previous studies and on the present study, smoking status might be an important factor explaining the disparate results in previous studies.

A few previous studies have concerned the association of M/L55 polymorphism with CHD. In a retrospective case-control study, there was no association between M/L55 polymorphism and CHD,[17] whereas a cross-sectional study suggested that the L55 allele increases the CHD risk in patients with type 2 diabetes.[10] In line with our results, the L55/L55 genotype was previously found to be a significant and independent predictor of carotid atherosclerosis by Schmidt et al.[29] Although the results from their study and ours are similar, there are differences between the 2 studies, eg, subject characteristics, atherosclerosis measurement, and study design. Unlike Schmidt et al,[29] we analyzed smokers and nonsmokers separately to see if smoking status changes the association between PON genotype and CAAD. It has also been shown that men have more plaque in their carotid arteries than women.[44,45] The subjects of a previous study were both men and women, whereas we studied only men. The significant association of PON L55/L55 with MMax IMT increases the reliability of our findings because MMax IMT, which is an average value of the IMT of 12 standard sites, gives a good picture of the involvement of early atherosclerotic changes in the whole carotid artery tree.[32] Because we measured all available arterial sites, including the internal carotid artery, a site with a high prevalence of atherosclerotic plaque, the relatively high cut-off limit (1.7 mm) for CAAD status prevented misclassification of subjects.

The observations from 2 previous studies,[10,29] as well as our study, are somewhat confusing because it was originally assumed that subjects with high PON activity (L55 homozygotes) toward paraoxon are better protected against atherosclerosis. Considering the relationship between PON and atherosclerosis, however, it is crucial to remember that the physiologic substrate of PON is still under discussion, although some candidates have been presented.[46] Therefore, the activity of PON, measured as its capacity to hydrolyze a nonphysiologic substrate, paraoxon, may not be adequate to predict the antioxidative properties of PON and consequently its role in CHD risk. This idea is supported by study results showing that HDL from subjects with the M55/M55 (low active) genotype protects LDL more effectively against peroxidation than HDL from subjects with the M55/L55 or L55/L55 genotype.[8] It has also emerged that enzyme with Q192 or M55 hydrolyzes diazoxon and the nerve gases sarin and soman faster than the high-activity enzyme with R192 or L55 allele.[47] In light of these studies, we cannot be sure if the enzyme with L55/L55 genotype is really highly active against the physiologic substrate of PON. We did not have suitable samples to measure PON concentration and activity to test the effect of genotype and smoking status on these parameters. However, the interpretation of these measurements seems to be unclear.

Recently, 3 promoter region polymorphisms of the human *PON1* gene have been described.[48] They have variable impact on promoter activity, which leads to differences in gene expression and serum concentration and activity of PON. The coding region M/L55 polymorphism also has a significant contribution to variation in serum PON concentration, whereas no significant contribution has been observed for the R/Q192 polymorphism.[10,48] This could be attributable in part to a strong association between the promoter polymorphisms and the M/L55 polymorphism. Thus it is possible that the promoter polymorphisms can influence the association between the M/L55 polymorphism and the risk of atherosclerosis.

In summary, our study of 199 randomly selected middle-aged men shows that the carotid IMT values of nonsmokers are significantly higher in L55 homozygotes than in M55 allele carriers. Our study does not provide information on whether M/L55 is the functional variant of PON but suggests that L55/L55 genotype associates with carotid atherosclerosis among nonsmokers. Furthermore, a significant PON M/L55 genotype–smoking status interaction was seen, and the effect of PON M/L55 genotype on the development of CAAD was almost opposite among smokers and nonsmokers. This suggests that the ability of PON to protect against CAAD is influenced by smoking, and the efficiency of this inhibition probably depends on the PON M/L55 genotype. This finding might explain the conflicting results of previous studies concerning the PON genotypes and atherosclerosis because smokers and nonsmokers are not usually analyzed separately.

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