Promoter Polymorphism T(–107)C of the Paraoxonase *PON1* Gene Is a Risk Factor for Coronary Heart Disease in Type 2 Diabetic Patients

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The serum enzyme paraoxonase (PON) protects LDLs from oxidative stress. We recently identified promoter polymorphisms of the PON gene that strongly affect gene expression and serum levels of the enzyme. The present study tested the hypothesis that promoter polymorphism T(–107)C could be a risk factor for vascular disease in type 2 diabetic patients by virtue of its ability to modulate serum concentrations of the antioxidant enzyme. The low-expressor genotype (TT) was associated with significantly lower serum PON concentrations, and it was over-represented in type 2 diabetic patients with coronary heart disease (CHD) (TT vs. TC+CC: odds ratio [OR] 1.64 [95% CI 1.03–2.61], $P < 0.05$). The association of the low-expressor genotype with an increased risk of disease was independent of other risk factors, including the coding region Q191R polymorphism (OR 2.12 [95% CI 1.19–3.70], $P = 0.01$). However, an interaction of the promoter polymorphism with the Q191R polymorphism, which was previously identified as an independent risk factor, was observed. The low-expressor promoter allele (–107T) associated with the high-risk 191R allele showed a lower-than-expected level of risk (OR 2.21 vs. the expected 4.76). The data are consistent with the hypothesis that low expression of the antioxidant enzyme PON increases the risk of CHD. Moreover, the promoter polymorphism appears to have a modulating effect on risk that is associated with the coding region polymorphism Q191R. This study indicates a strong genetic component to the antioxidant capacity of HDLs. *Diabetes* 49:1390–1393, 2000

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CHD, coronary heart disease; D*, maximum likelihood estimate of linkage disequilibrium; OR, odds ratio; PON, paraoxonase.

The hypothesized role of the serum enzyme paraoxonase (PON) is to protect LDLs from oxidation (1). Substantial in vitro evidence supports this contention (2,3), which is complemented by data from animal models (4,5). In particular, studies of PON knockout mice have revealed that absence of serum PON activity is associated with greater susceptibility of lipoproteins to oxidation and formation of more extensive atherosclerotic plaques (5). One extrapolation from these studies is that decreased serum PON will increase the risk of LDL oxidation and, consequently, the risk of vascular disease, given the preponderant atherogenic role attributed to oxidized LDL (6,7). In earlier studies, we identified a coding region polymorphism (Q191R) of the *PON1* gene (affecting exogenous enzyme activity) as an independent risk factor for coronary disease in type 2 diabetes. Recently, we demonstrated that a second coding region polymorphism of the *PON1* gene (L54M) correlated with variations in serum levels of the enzyme (8). Subsequently, we showed differences in the levels of expression of L- and M-type mRNAs by human liver; this finding was compatible with higher serum concentrations of L-type PON protein (9). In a continuation of the studies, we cloned the promoter region of the *PON1* gene and identified 3 polymorphic sites: T(–107)C, G(–824)A, and G(–907)C. Genotyping studies in a healthy population established that the polymorphisms were frequent and that they had a strong impact on gene expression and serum concentrations, in particular the T(–107)C site (10). By virtue of its influence on serum PON levels, we hypothesized that promoter polymorphism T(–107)C could be a risk factor for vascular disease. The hypothesis has been tested in a type 2 diabetic population.

The groups with and without coronary heart disease (CHD) (Table 1) have been described previously (11). Age and duration of diabetes were significantly higher in the CHD group, which also had a higher percentage of men. HDL cholesterol was significantly lower in the patients with CHD. There was a trend to lower serum concentrations of PON in the group with CHD, and this trend was confirmed by a significantly lower enzyme activity ($P = 0.038$) (Table 1). Table 2 shows the serum PON concentrations and activities as a function of the T(–107)C promoter polymorphism for the combined populations. There were highly significant differences between the genotypes. Comparable results (data not

TABLE 1
Demographic and clinical characteristics of type 2 diabetic patients with and without CHD

Parameter	Patients with CHD	Patients without CHD	*P*
Age (years)	62.8 ± 9.6	59.0 ± 10.5	<0.001
Sex (M/F)	104/33	175/98	0.01
BMI (kg/m^2)	27.5 ± 4.1	27.9 ± 4.6	0.37
Diabetes duration (years)	15.7 (0.8)	13.4 (0.5)	0.01
Smoking status (%) (never smoked/ex-smoker/active smoker)	41/45/14	67/21/12	<0.001
Systolic blood pressure	145.9 ± 20.3	143.2 ± 19.8	0.20
Diastolic blood pressure	83.4 ± 11.6	81.3 ± 9.9	0.058
Cholesterol (mmol/l)	6.0 ± 1.1	5.9 ± 1.1	0.40
Triglycerides (mmol/l)	1.85 ± 0.9	1.7 ± 1.1	0.054
HDL cholesterol (mmol/l)	1.18 ± 0.3	1.3 ± 0.4	0.031
LDL cholesterol (mmol/l)	4.21 ± 1.24	4.00 ± 1.15	0.062
PON mass (µg/ml)	79.2 ± 23.6	83.9 ± 25.0	0.13
PON activity (U/ml)	11.1 ± 2.7	12.2 ± 3.3	0.038
LDL cholesterol:PON mass	0.059 ± 0.02	0.052 ± 0.02	0.01
Genotypes T(–107)C (TT/CT/CC)	42/70/25	58/152/63	0.09* <0.05†
Alleles (T/C)	154/120	268/278	0.06
Genotypes L54M (LL/LM/MM)	59/52/26	94/139/40	<0.05
Genotypes Q191R (QQ/QR/RR)	58/67/12	140/118/15	0.16

Date are *n*, means ± SD, or means ± SE (for duration). PON activity is given for phenylacetate. LDL cholesterol was estimated by the Friedewald equation. *Comparison among all 3 groups; †comparison between TT and (CT+CC).

shown) were obtained for the populations analyzed individually. There were no significant differences in specific enzyme activities for either substrate, which was consistent with an effect of the promoter polymorphism on protein concentrations alone. The genotype frequencies were in Hardy-Weinberg equilibrium. Linkage disequilibrium (12) was observed between the promoter polymorphism and the previously described coding region polymorphism L54M (maximum likelihood estimate of linkage disequilibrium [D*] = –0.055; χ^2 = 11.8; $P < 0.001$). No linkage was observed between T(–107)C and the Q191R polymorphism (D* = –0.012; χ^2 = 0.54). Analysis of promoter genotype frequencies in the CHD subgroups showed a greater percentage of type 2 diabetic patients with the TT genotype (42%) compared with the CC genotype (28.4%) in the group with CHD, thus indicating an association of the –107T polymorphism with CHD (TT vs. TC+CC: odds ratio [OR] 1.64 [95% CI 1.03–2.61], $P < 0.05$). The data were further examined by logistic regression analysis (Table 3). The promoter polymorphism was associated with the presence of CHD independently of other risk factors, most notably sex; triglyceride, cholesterol, and HDL cholesterol levels; age; and the coding region polymorphism Q191R. Given the previously documented association between the R allele of the 191 polymorphism and the increased risk of vascular disease, an analysis restricted to subjects who were homozygous for the Q allele of the polymorphism was also performed. Figure 1 shows the distribution between the groups with and without CHD according to the promoter polymorphism. A greater percentage of subjects with the low-expressor TT genotype were present in the group with CHD compared with patients with higher-expressor CT and CC genotypes (OR 2.63 [95% CI 1.30–5.25], $P < 0.01$). This was confirmed by significantly lower enzyme activity in the group with CHD (Table 1). A final analysis (13) concerned the joint effects of the promoter and Q191R polymorphic sites. Table 4 shows the ORs in subgroups defined by CHD and the polymorphisms, with the CC+CT/QQ subgroup serving as

TABLE 2
Serum PON concentrations and activities as a function of the promoter T(–107)C polymorphism

Genotype	Concentration (µg/ml)	Enzyme activity (U/ml)	
		Phenylacetate	PON
CC	97.5 ± 27.8	71.7 ± 13.1	212.3 ± 113.6
CT	83.3 ± 21.5	59.7 ± 12.7	162.0 ± 85.9
TT	63.9 ± 17.0	45.1 ± 12.2	125.7 ± 87.0
P	<0.0001	<0.0001	<0.0001

Data are means ± SD, unless otherwise indicated.

TABLE 3
Logistic regression analysis of determinants of CHD

Parameter	OR	95% CI	*P*
Age	1.04	1.01–1.07	<0.01
Sex	1.44	1.02–1.80	0.03
HDL cholesterol	0.33	0.16–0.68	0.003
Polymorphism T(–107)C TT vs. (TC+CC)	2.12	1.19–3.70	0.01
Polymorphism Q191R (QR+RR) vs. QQ	1.92	1.04–2.83	0.03

Other parameters tested in the model were triglyceride levels, cholesterol levels, blood pressure measurements, duration of diabetes, and smoking status.

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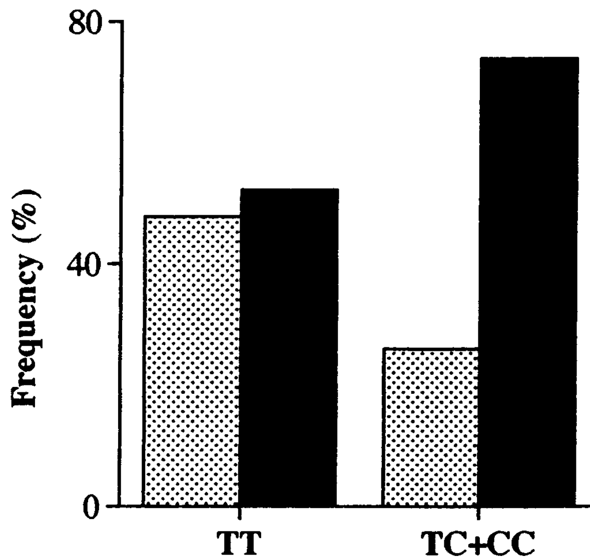

Fig. 1. The percentage of the promoter genotypes TT and (TC+CC) in the CHD-positive (▨) and CHD-negative (■) subgroups for type 2 diabetic patients homozygous for the 191Q allele. *P* < 0.01.

the reference group. As previously mentioned, compared with the reference group, the TT/QQ group showed an increased risk. However, overexpression of the R allele (CC+CT/QR+RR) was also associated with an increased risk (OR 1.81), even though the TT/QR+RR subgroup showed an OR (2.25) less than the expected value (4.76). This suggests an interaction between the 2 polymorphisms.

The present study has identified a polymorphism in the promoter region of the *PON1* gene as an independent genetic risk factor for CHD in type 2 diabetic patients. The polymorphism is associated with significant differences in the expression of a reporter gene in cell transfection studies (10). In this study and the study by Leviev and James (10), these differences correlated with variations in serum concentrations and activities of PON. The –107T allele is consistently associated with lower PON expression, lower serum concentrations, and lower activities of the enzyme. Moreover, in the present study, it is an independent risk factor for CHD. The data are thus consistent with the hypothesis that low expression of the enzyme represents a risk factor for CHD in type 2 diabetes. According to this hypothesis, low PON expression constitutes a pathophysiological mechanism, compatible with in vitro and in vivo data and with the role of PON as an antioxidant, that protects LDLs from lipid peroxidation. In this context, it should be noted that the LDL cholesterol–to–PON concentration ratio was significantly higher in the group with CHD; this finding was consistent with a reduced capacity of PON to limit LDL oxidation. It has been suggested that diabetic patients are at an increased risk for oxidative stress (14). This may be due, in part, to particular features of the diabetic state, notably hyperglycemia and advanced glycosylation end products. Mechanisms have been proposed by which both could initiate and accentuate lipid oxidation (15,16). In this context, a reduced antioxidant capacity represented by lower serum PON concentrations assumes particular relevance. In preceding studies, we (8,11) and others (17–19) identified the coding region 191 polymorphism as a risk factor for vascular disease. The higher-risk R191 allele has modified activity toward exogenous substrates, although the physiological relevance of the activity polymorphism has not been clarified. The association of CHD with the promoter polymorphism is independent of the effect of the 191 polymorphism. This was demonstrated by multivariate analysis in which both polymorphisms entered the regression model. Moreover, when the analysis was restricted to noncarriers of the R allele, the effect of the promoter polymorphism was maintained. It should also be noted that there is no linkage between the 2 polymorphisms. However, the present study suggests a complex interaction between the 2 polymorphisms. In the presence of the R allele, previously identified as a high-risk allele (11), lower expression (–107T allele) is associated with a lower-than-expected level of risk. This observation is consistent with the promoter and 191 polymorphisms influencing risk by distinct mechanisms, during which underexpression of the R allele may limit its own pathological influence. It should be noted that overexpression of the R allele (CC+CT/QR+RR) was also associated with increased risk (Table 4). In this context, it is particularly important to define more precisely the influence of the R allele enzyme activity on physiological substrates. One implication of these results is that the promoter polymorphisms may be confounding factors in analyses that have focused on the Q191R polymorphism. In several studies, the Q191R polymorphism failed to emerge as an independent risk factor for vascular disease (20). Given our present data, it would be interesting to determine the influence of the promoter polymorphism in these populations. Finally, our data underline the genetic influence on the serum antioxidant capacity of diabetic patients, a population that appears particularly susceptible to oxidative stress.

RESEARCH DESIGN AND METHODS

Type 2 diabetic patients were recruited and characterized with respect to the presence or absence of CHD, as described previously in detail (11). Briefly, the

TABLE 4
Relative ORs as a function of CHD and the T(–107)C and Q191R polymorphisms

Genotype subgroup	Patients with CHD	Patients without CHD	OR (95% CI)
CC+CT/QQ	35	112	1.0 (reference)
TT/QQ	23	28	2.63 (1.32–5.25)
CC+CT/QR+RR	60	106	1.81 (1.08–3.05)
TT/QR+RR	19	27	2.25 (1.07–4.72)

Data are *n*, unless otherwise indicated. The subgroups were defined according to the T(–107)C and Q191R genotypes (χ^2 = 11.04, P < 0.025). The relative amplitude of interaction was –0.95%.

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group with CHD had confirmed transmural myocardial infarction or positive coronarogram (>70% narrowing in at least one major artery). The group without CHD was selected from the CEPH type 2 diabetes family register; these subjects had no history of angina pectoris and had a normal resting electrocardiogram. The study was conducted according to the requirements of the Ethics Committee of Geneva University.

Serum PON enzyme activities and concentrations were determined as previously described (8). Other serum parameters were analyzed as previously reported (8,11). The coding region polymorphisms Q191R and L54M were determined by restriction isotyping (8). The promoter polymorphism T(–107)C was analyzed by allele-specific hybridization (10).
Categories were compared between groups by use of the χ^2 test and crude OR. Allele frequencies were estimated by the allele-counting method, and Hardy-Weinberg's equilibrium was tested by the χ^2 test. Multivariate analyses were performed with a logistic regression model adjusted for all variables.

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