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Association of Paraoxonase 1 Gene Polymorphism with Intima-Media Thickness (IMT) of the Carotid Arteries in Japanese Type 2 Diabetic Patients

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Abstract: Purpose: We investigated the association between paraoxonase 1 (PON1)-192 genotypes and intima-media thickness (IMT) of carotid arteries in diabetic patients.

Methods: One hundred and fifty-five Japanese type 2 diabetic patients aged from 40 to 79 years were enrolled in this study. Genotypes of the patients were determined using the PCR-RFLP method. The IMT of carotid arteries of the subjects was measured by ultrasound imaging.

Results: The PON1 genotypes frequencies were as follows: 18QQ (0.116), 70QR (0.452) and 67RR (0.432). IMT values of the RR group were significantly greater (1.08±0.41 mm, n=67) than those of the Q group, which consisted of patients carrying one or two Q alleles (0.95±0.27 mm, n=88, *P*=0.023). There were no significant difference in the clinical characteristics between the two groups.

Conclusion: The results indicate that the PON1-192RR genotype is associated with intima-media thickening of the carotid arteries in diabetic patients.

Key words: paraoxonase 1, genetic polymorphism, intima-media thickness (IMT)

Introduction

Serum paraoxonase 1 (PON1) is associated with plasma high-density lipoprotein, and prevents the oxidative modification of low-density lipoprotein[1)]. PON1 knockout mice are highly susceptible to developing atherosclerosis compared to wild-type control mice[2)]. Transgenic mice overexpressing PON1 display decreased atherosclerotic lesions[3)]. The serum PON1 concentration in relation to PON1-192 genetic polymorphism is significantly lower in the RR (Arg/Arg) than in the QQ (Gln/Gln) genotype[4)]. In Caucasian and Japanese diabetic patients, the R allele

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Table 1 Clinical characteristics of the PON1-192 genotype groups

Characteristics	Genotype and *P*-value		
	RR group	Q group (QR and QQ genotypes)	*P*-value
N	67	88(QR:70, QQ:18)	
Sex (M/F)	44/23	53/35	0.488[a]
Age (years)	60.7±8.7	60.8±8.9	0.971[b]
Diabetes duration (years)	9.9±8.8	11.7±8.7	0.128[c]
Body mass index (kg/m^2)	23.6±3.2	23.6±3.1	0.968[b]
HbA1c (JDS) (%)	8.4±2.7	7.8±1.9	0.160[c]
Fasting glucose (mg/dl)	201.7±102.6	174.1±68.6	0.301[c]
Total cholesterol (mg/dl)	202.7±37.1	202.2±36.1	0.930[b]
HDL cholesterol (mg/dl)	52.5±14.1	54.6±14.7	0.934[c]
LDL cholesterol (mg/dl)	120.7±32.6	119.3±33.5	0.799[b]
Triglycerides (mg/dl)	144.8±85.6	153.2±113.4	0.475[c]
Creatine (mg/dl)	0.849±0.314	0.885±0.537	0.506[c]
Systolic blood pressure (mmHg)	139.4±18.6	143.9±23.0	0.200[b]
Diastolic blood pressure (mmHg)	80.5±10.7	80.8±12.7	0.893[b]

Data are the mean±S.D. $P<0.05$ was regarded as significant.
[a] χ^2-test
[b] Student's t-test
[c] Mann-Whitney's U test

increases the ischemic heart disease risk[5-8]. However, Bhattacharyya T. *et al*. reported that the Q allele increases major adverse cardiac events in patients undergoing diagnostic coronary angiography[9]. Thus, the relation between PON1-192 genetic polymorphism and atherosclerosis is controversial. Here, we investigated the relation of the carotid artery IMT with genotypes of PON1 in Japanese diabetic patients, and examined their clinical data, because IMT values of the carotid arteries correlate with the progression of systemic atherosclerosis.

Subjects and Methods

1. Study subjects

The study subjects were 155 consecutive Japanese patients with type 2 diabetes aged from 40 to 79 years who were treated at our hospital. These subjects had no history of major cardiovascular events and did not suffer from severe nephropathy. Written, informed consent was obtained from the subjects who agreed to participate in the study, and the study was approved by the institutional review committee. Since the QQ group was very small, the subjects were divided into two groups based on the genotype of PON1. One group consisted of patients carrying two R alleles (RR group) and the other group consisted of patients carrying one or two Q alleles (Q group). The characteristics of the subjects are presented in Table 1.

2. Genotyping of PON1-192

Genomic DNA was extracted and purified from peripheral blood with the QIAamp Blood Kit (QIAGEN, Germany). For the genotyping of PON1, we used the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. We used the primers described by Kujiraoka *et al*.: 5'-TAT TGT TGC TGT GGG ACC TGA G-3'(sense primer) and 5'-CTT GCC ATC GGG TGA AAT GTT G-3' (antisense primer)[4]. PCR was performed with 30 cycles of amplification with annealing at 60°C. The PCR products (193 bp) were digested with the *AlwI* restriction enzyme, separated by 3.0% agarose gel electrophoresis, and visualized using ethidium bromide. Genotype Q (Gln192) corresponded to 193-bp fragments and genotype R (Arg192) corresponded to 63- and 130-bp fragments.

3. Laboratory measurements

Fasting blood samples were drawn from the subjects, and

Table 2 Genotype and allele distribution of the study subjects

Genotype	Frequency (n=155)
QQ	0.116 (18)
QR	0.452 (70)
RR	0.432 (67)

Allele	Frequency (n=310)
Q	0.342 (106)
R	0.658 (204)

serum total and HDL cholesterol, triglyceride, creatinine, plasma glucose, and HbA_{1c} (JDS) levels were determined by the clinical research center of our hospital, following standard laboratory protocols. LDL cholesterol values were calculated using the Friedewald equation.

4. B-mode ultrasonography

We used B-mode ultrasound imaging with 7.5-MHz transducers giving an axial resolution of 0.1 mm (apparatus, Toshiba SSA-370A; probe, PLM-703AT). All examinations were performed by a single trained sonographer. The IMT measurements were conducted as described in a previous report[10]. The IMT of the far wall was measured on a longitudinal scan of the common carotid arteries at a point about 10 mm proximal to the beginning of the dilation of the bulb. We chose an area that was smooth, and avoided areas of focal thickening. We measured the IMT value of the left and right carotid arteries in each patient, and the average IMT value was used as a representative value for each individual in the statistical analysis.

5. Statistical analysis

Data are expressed as the mean ± S.D. The normality of continuous variables was checked. When the data showed a normal distribution with equal variance, we used Student's t-test. When the data showed a normal distribution with an unequal variance, we used Welch's t-test. When the data did not show a normal distribution, we used Mann-Whitney's U test. Values of $P<0.05$ were regarded significant. We performed the analyses using Statcel 2 (OMS Publishing Inc., Japan).

Results

1. Characteristics of the study subjects

There were no significant differences in the clinical characteristics of the RR and Q groups.

Table 3 PON1-192 genotypes and IMT values of both genotypes in type 2 diabetic patients

	Genotype and *P*-value		
	RR	Q group (QR+QQ)	*P*-value
N	67	88 (70+18)	
IMT (mm)	1.08±0.41	0.95±0.27	0.023

Data are the mean±S.D. $P<0.05$ was regarded as significant. The *P*-value was calculated by Welch's t-test.

2. Genotyping of PON1-192

The genotypes were determined using the PCR-RFLP method. The sequenced PCR products were consistent with the reported data (data not shown). The results of the genotypes are listed in Table 2. The PON1 genotypes frequencies were as follows: 18QQ (0.116), 70QR (0.452) and 67RR (0.432). The prevalence of the Q allele was 0.342 and that of the R allele was 0.658, comparable to those of healthy Japanese volunteers studied previously[4]. The distribution of PON1-192 genotypes showed Hardy-Weinberg equilibrium.

3. IMT measurements

The IMT values are shown in Table 3. IMT of the RR group was significantly greater (1.08±0.41 mm, n=67) than that of the Q group (0.95±0.27 mm, n=88, $P=0.023$), although there was no significant difference between the two groups in clinical characteristics (Table 1).

Discussion

This study investigated the association between the PON1-192 genotypes and IMT values of carotid arteries, a measure that indicates the progression of systemic atherosclerosis. Our results indicate that the PON1-192 RR

genotype may be associated with intima-media thickening of carotid arteries. Kujiraoka *et al*. reported that the RR group had a higher paraoxonase activity[4]. Also Aviram reported that PON1 has phospholipase A2-like activity, and it could form lysophosphatidylcholine (LPC) from phosphatidylcholine[11]. LPC exhibits marked atherogenic activity[12-13]. PON1-192 RR may have a larger amount of phospholipase A2-like activity, by which LPC might be formed. Thus, a larger atherogenic LPC might advance intima-media thickening of carotid arteries in PON1-192 RR patients. However, appropriate experiments are needed to test this hypothesis. Hu et al. reported that, in Chinese diabetic patients, IMT values of carotid arteries were significantly greater in the RR group than in both the QR and QQ groups[14]. Their findings correspond with ours, but total cholesterol and LDL cholesterol values in the RR group were significantly higher than those in the QR and QQ groups in their study. High LDL cholesterol values usually cause systemic atherosclerosis. High total cholesterol and LDL cholesterol values would explain intia-media thickening of the carotid arteries. In our results, the clinical characteristics of the subjects did not show any significant differences. Thus, we conjecture that genetic polymorphism of PON1-192 may be a major cause of increased IMT values in the carotid arteries. The RR group of our study had longer diabetic duration (9.9±8.8 years) and higher HbA_{1c} values (8.4±2.7 %) than those of the Chinese RR group (7.9±4.0 year, 7.56±1.5 %). Namely, our study subjects had been more strongly affected by the diabetic condition. Diabetes is major cause of atherosclerosis. Luersen reported that a group of healthy males with the PON1-192QR polymorphism had a higher pro-inflammatory cytokine TNF-alpha level than a QQ group[15]. TNF-alpha has atherogenic effects[16]. Thus, the PON1-192 RR genotype may be associated with intia-media thickening of the carotid arteries. However, our findings need to be confirmed in further studies, especially in a larger study population.

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