PON1 polymorphism, diabetes mellitus, obesity, and risk of myocardial infarction: Modifying effect of diabetes mellitus and obesity on the association between PON1 polymorphism and myocardial infarction

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Purpose: Previous studies on *PON1* gene polymorphism and the risk of coronary atherosclerotic diseases have been inconsistent. This may be in part due to population difference in prevalence of high oxidative stress and its modifying effect on the association. Diabetes and obesity are two major risk factors of myocardial infarction (MI) and associated with high oxidative stress. We investigated the association between *PON1* Q192R polymorphism and the risk of myocardial infarction (MI) and in particular, whether the association can be modified by diabetes mellitus (DM) and obesity. **Methods:** We conducted a case-control study, including 154 MI cases and 154 controls enrolled in Beijing, China. Epidemiological and clinical data and *PON1* Q192R genotype were obtained from each subject. **Results:** Without considering the modifying effect of DM and obesity, *PON1* Q192R polymorphism was not associated with MI. When simultaneously examining the joint association of this polymorphism, DM, and obesity with MI, as compared to subjects with QQ genotypes and without DM and obesity, subjects with QR/RR genotypes and with either DM or obesity had significantly higher risk of MI (OR = 3.6, 95% CI: 1.5–8.7). **Conclusion:** Our data suggest that *PON1* Q192R polymorphism was not independently associated with MI but further increased the risk of MI among the subjects with DM, obesity, or both, the conditions associated with high oxidative stress. ***Genet Med*** 2005:7(1):58–63.

Key Words: *human paraoxonase 1 gene (*PON1*), polymorphism, myocardial infarction, diabetes mellitus, obesity.*

Coronary atherosclerotic disease (CAD), including its most severe form, myocardial infarction (MI), is the leading cause of death in many countries and is estimated to be the single largest cause of disease burden globally by the year 2020.[1] Historically, CAD is much less common in China than in Europe and the USA. However, in recent years the prevalence of CAD in China has been on the rise, especially in the major cities.[2] This upward trend may be in part due to a shift from traditional Chinese diet to a Westernized diet and decreased physical activity. In addition to environmental risk factors, there is growing evidence that genetic factors play an important role in predisposition to

CAD.[3] As lipoprotein oxidation is a key initial factor for atherosclerosis, genes involved in this process are good candidate genes for CAD. This study focused on human serum paraoxonase 1 (*PON1*) gene, encoding a HDL associated enzyme that hydrolyzes paraoxon and prevents low-density lipoprotein (LDL) oxidation.[4,5]

A number of studies have investigated the *PON1* Q192R polymorphism in relation to the risk of CAD. A recent meta-analysis of 43 studies showed a weak overall association between the *PON1* Q192R polymorphism and CAD. However, the results of these studies have been inconsistent across populations.[7–15] The R allele is associated with CAD in US Caucasians,[7] in Asian Indians,[8,9] and in Japanese,[10] but not in French,[11] Italians,[12] Finns,[13] Koreans,[14] and in Chinese living in Singapore[8] and Taiwan.[15] The reasons for the inconsistent findings may be multiple, including small sample size, multiple comparisons, population stratification, and inadequate consideration of confounders and gene-environmental interactions.[16] Another possibility is the study population difference in the prevalence of high oxidative stress, for example, diabetes mellitus (DM) and obesity. DM and obesity are two components of metabolic syndrome and major risk factors of CAD. It

has been shown that lipid peroxide concentrations are significantly higher in metabolic syndrome subjects than in non-metabolic syndrome subjects.[17] Moreover, *PON1* Q192R polymorphism has been consistently associated with CAD among subjects with type 2 diabetes,[18–21] a condition with high oxidative stress.

We conducted a case-control study in Beijing, China, to investigate the association between *PON1* Q192R polymorphism and the risk of MI and whether the association can be modified by DM and obesity.

METHODS

Study population

Study subjects were enrolled from 1996 to 1998 in Beijing, China. All subjects gave informed consent, and the study protocol was approved by the Institutional Review Board of the Health Science Center of Peking University and Harvard School of Public Health.

A total of 156 MI cases with an age range of 32 to 72 years were enrolled from the Department of Cardiology, the Peking University First Affiliated Hospital. All the cases were admitted to the hospital and diagnosed with acute MI according to the criteria of World Health Organization (WHO). A total of 156 age- (within 5 years) and sex- matched controls were enrolled from the hospitalized noncardiac patients in the other departments of the same hospital, including Pulmonary Diseases, Digestive Diseases, General Surgery, Orthopedics, and Urology. All the controls had no history of CAD and showed normal findings on cardiac physical examination, electrocardiogram, and echocardiography. As there were 4 body mass index (BMI) data missing (2 in cases and 2 in controls), in the subsequent analysis we just focused on the 154 cases and 154 controls with full demographic information.

Epidemiological and clinical data

A standard questionnaire was administered by a trained interviewer to obtain information on each subject's history of physician-diagnosed diseases, including hypertension, hyperlipidemia, and diabetes mellitus, and environmental exposures, including cigarette smoking and alcohol consumption. Height and weight were measured by trained examiners after each subject removed shoes and outer wear; height was measured to the nearest 1 mm on a portable stadiometer, and weight was measured to the nearest 0.1 kg with the subject standing motionless on the scale. BMI was calculated as weight (kg)/height^2 (m^2). Obesity is defined as BMI > 27 kg/m^2 according to the suggested criteria of obesity for Asian populations.[22] Cigarette smokers were defined as those who smoked at least one cigarette per day and had smoked more than 1 year. Ever smokers included both current smokers and former smokers who had quit smoking. Alcohol drinkers were defined as those who drank at least two times a week and had drunk for more than 1 year. Ever drinkers include current drinkers and former drinkers who had quit drinking. Hypertension was defined as systolic blood pressure ≥ 140 mm Hg and/or diastolic blood pressure ≥ 90 mm Hg, or treatment with antihypertensive medication. Diabetes mellitus was defined as fasting blood glucose levels above 110 mg/dL, or treatment with antidiabetic medication. Hyperlipidemia was defined as total cholesterol (TCHO) > 5.1 mmol/L and/or low-density lipoprotein (LDL) > 3.1 mmol/L or currently taking lipid lowing medications.

Genotyping methods

The genomic DNA was isolated from white blood cells by a previously described DNA extraction methods.[23] *PON1* Q192R genotype was determined by PCR amplification of genomic DNA followed by restriction-endonuclease digestion. The detailed method has been published elsewhere.[7]

Statistical analysis

Continuous variables for cases and controls were expressed as mean and standard error (SE) and were compared using Student's *t* test. Categorical variables were presented as counts and percentages and were compared by chi-square test. The concordance of the genotype frequency distribution with the Hardy-Weinberg equilibrium was evaluated by a chi-square test. The association of *PON1* Q192R polymorphism with the risk of MI was investigated sequentially. First, we investigated whether the Q192R Polymorphism is an independent risk factor of MI. This polymorphism has three possible genotypes: QQ, QR, and RR. A chi-square test was performed to compare the genotype distribution among the cases and controls. Then a logistic regression analysis was performed to estimate odds ratio (OR) and 95% confidence interval (CI) of MI by comparing individuals taking different genotypes. The logistic regression analysis adjusted for age (as a continuous variable), sex, BMI (as a continuous variable), cigarette smoking (ever, never), alcohol consumption (ever, never), and history of physician-diagnosed hypertension (yes, no), diabetes mellitus (yes, no), and hyperlipidemia (yes, no). We evaluated 4 possible genetic models: Dominant (QQ = 0, QR = 1, and RR = 1); recessive (QQ = 0, QR = 0, and RR = 1); additive (QQ = 0, QR = 1, and RR = 2); and no restriction (no assumption made). We found that the risk of MI was similar between QR and RR genotype, but differed between QQ and QR genotype. Thus, our data suggested an additive or dominant role for the R allele (or a recessive role for the Q allele). In the subsequent analysis of joint association of genotype and other risk factors of MI, we combined QR and RR genotypes together, and used QQ genotype as reference group.

We further investigated whether the association between *PON1* Q192R polymorphism and MI can be modified by the conditions of DM and obesity while adjusting for other important covariates including hypertension, hyperlipidemia, and cigarette smoking. We first examined the joint association of *PON1* Q192R genotype and each of the two conditions with MI, in which the subjects were divided into four groups according to the genotype (QQ, QR/RR) and the condition: DM (yes, no), or obesity (BMI < 27, BMI ≥ 27). Then, we examined the joint association of genotype and both of the two conditions with MI. We compared six groups defined by geno-

type (QQ, QR/RR) and presence of the two conditions (neither obesity nor DM, either obesity or DM, both obesity and DM). We also tested potential interaction between genotype and DM and between genotype and obesity using a multivariate logistic regression model by including a product term of *PON1* Q192R polymorphism × DM and the *PON1* Q192R polymorphism × obesity. All the statistical analyses were performed using SAS statistical software, version 6.12. A value of *P* < 0.05 was considered to be statistically significant on two-sided test.

RESULTS

The demographic and clinical characteristics of cases and controls are summarized in Table 1. As compared to control

Table 1
Characteristics of the cases and the controls, Beijing, China

Variable	Controls ($n = 154$)	Cases ($n = 154$)
Age (years)	56.2 ± 9.3	56.3 ± 9.2
BMI (Kg/m²)	23.9 ± 3.1	25.6 ± 3.1†
Sex		
Male	124 (80.5)	123 (79.9)
Female	30 (19.5)	31 (20.1)
History of hypertension		
No	119 (77.3)	81 (52.6)†
Yes	35 (22.7)	73 (47.4)
History of Diabetes Mellitus		
No	145 (94.2)	114 (74.0)†
Yes	9 (5.8)	40 (26.0)
History of Hyperlipidemia		
No	147 (95.5)	107 (69.5)†
Yes	7 (4.5)	47 (30.5)
Obesity		
No	132 (85.7)	101 (65.6)†
Yes	22 (14.3)	53 (34.4)
Smoking status		
Never	47 (30.9)	38 (25.3)
Ever	105 (69.1)	112 (74.7)
Alcohol drinking		
Never	103 (66.9)	108 (70.1)
Ever	51 (33.1)	46 (29.9)
PON1 Q192R genotype		
QQ	31 (20.1)	27 (17.5)
QR	73 (47.4)	66 (42.9)
RR	50 (32.5)	61 (39.6)

Continuous variables are expressed as mean ± standard error and categorical variables are expressed as counts and percentage; obesity was defined as BMI ≥ 27; **P* < 0.05, †*P* < 0.01.

group, case group had a significantly higher level of BMI and higher proportion of history of physician diagnosed hypertension, DM, hyperlipidemia, and obesity. The respective frequencies of Q (Gln) and R (Arg) alleles for the *PON1* Q192R polymorphism were 0.44 and 0.56 among controls and 0.39 and 0.61 among cases. Genotype frequencies were concordant with Hardy-Weinberg equilibrium among both cases ($\chi^2_1 = 1.38$, $P = 0.24$) and controls ($\chi^2_1 = 0.23$, $P = 0.63$). It is noted that the frequency of R allele is higher in the cases than in the controls; however, the difference was not statistically significant.

Table 2 presents genotype frequency among cases and controls, and adjusted association of *PON1* Q192R polymorphism with the risk of MI for the total sample and stratified by obese and DM status. As compared to the subjects with QQ genotype (the reference group), the adjusted OR for subjects with QR genotype, RR genotype, and combined QR and RR genotypes (QR/RR) were 1.3 (95% CI: 0.6–2.6), 1.4 (95%CI: 0.7–3.0), and 1.3 (95% CI: 0.7–2.6), respectively. It is noted that OR differs in obesity and DM strata, with obese and DM subjects had greater OR than nonobese or non-DM subjects.

Table 3 presents the joint association of *PON1* Q192R genotype and obesity with the risk of MI. As compared to the nonobese subjects with QQ genotype (the reference group), the obese subjects with QR/RR genotype had the highest risk of MI: OR = 2.9 (95%CI: 1.2–7.1, *P* = 0.02). A test of interaction between the genotype and obesity by including their product term in the logistic regression model was not statistically significant (OR = 1.4, 95% CI: 0.3–7.5, *P* = 0.71, data available upon request). Similarly, Table 4 presents the joint association of *PON1* Q192R genotype and DM with the risk of MI. As compared to the non-DM subjects with QQ genotype (the reference group), the DM subjects with QR/RR genotype had the highest risk of MI: OR = 8.4 (95%CI: 2.5–28.0, *P* < 0.01). A test of interaction between the genotype and DM by including their product term in the logistic regression model was suggestive but not statistically significant (OR = 3.5, 95% CI: 0.6–21.4. *P* = 0.22, data available upon request). Based on the results from Tables 3 and 4, the modifying effect of diabetes appears to be greater than obesity.

Table 5 presents the joint association of *PON1* Q192R genotype, DM and obesity with the risk of MI. As compared to subjects without DM and obesity and with QQ genotypes (reference group), there is a trend of increased risk of MI among subjects with the presence of either DM or obesity (OR = 1.9, 95% CI: 0.5–7.3, *P* = 0.34) and both (OR = 4.5, 95% CI: 0.4–55.2, *P* = 0.24). On the other hand, QR/RR genotype alone in the absence of DM and obesity was not associated with increased risk of MI (OR = 1.1, 95% CI: 0.5–2.3, *P* = 0.89). In the presence of either DM or obesity, the risk of MI for subjects with QR/RR genotype was significantly increased (OR = 3.6, 95% CI: 1.5–8.7, *P* < 0.01). The highest risk of MI was found among subjects with QR/RR genotype and with both DM and obesity (OR = 13.6, 95% CI: 1.5–124.8, *P* = 0.02).

Table 2
Association of *PON1* Q192R polymorphism with the risk of MI in all the subjects and in subjects stratified by obesity and diabetes

Genotype	No. of Subjects (%)		Adjusted[a]		
	Controls	Cases	OR	95% CI	*P*
All					
QQ	31 (20.1)	27 (17.5)	1.0		
QR	73 (47.4)	66 (42.9)	1.3	0.6–2.6	0.50
RR	50 (32.5)	61 (39.6)	1.4	0.7–3.0	0.35
QR/RR	124 (80.5)	129 (83.8)	1.3	0.7–2.6	0.39
Obese					
QQ	4 (18.2)	9 (17.0)	1.0		
QR/RR	18 (81.8)	44 (83.0)	1.4	0.3–5.7	0.65
Nonobese					
QQ	27 (20.5)	18 (17.8)	1.0		
QR/RR	105 (79.5)	83 (82.2)	1.1	0.6–2.4	0.72
DM					
QQ	4 (44.4)	7 (17.5)	1.0		
QR/RR	5 (55.6)	33 (82.5)	5.7	0.8–42.5	0.09
Non-DM					
QQ	27 (18.6)	20 (17.5)	1.0		
QR/RR	118 (81.4)	94 (82.5)	1.2	0.6–2.4	0.63

[a]Adjusted OR was calculated by adjusting age, sex, history of physician diagnosed hypertension, and hyperlipidemia, cigarette smoking, and alcohol consumption.

Table 3
Joint association of *PON1* Q192R polymorphism and obesity with the risk of MI

Genotype	Obesity	No. of Subjects (%)		Adjusted[a]		
		Controls	Cases	OR	95% CI	*P*
QQ	No	27 (17.5)	18 (11.7)	1.0		
QQ	Yes	4 (2.6)	9 (5.8)	1.6	0.3–7.9	0.53
QR/RR	No	105 (68.2)	83 (53.9)	1.3	0.6–2.6	0.53
QR/RR	Yes	18 (11.7)	44 (28.6)	2.9	1.2–7.1	0.02

[a]Adjusted OR was calculated by adjusting age, sex, history of physician diagnosed hypertension, diabetes, and hyperlipidemia, cigarette smoking, and alcohol consumption.

DISCUSSION

PON1 has received considerable attention in the last decade because of its potential role in antioxidation of LDL. So far at least three polymorphisms have been investigated in relation to CAD. Among them, only the polymorphism of Q192R was found to have a weak association with CAD in a recent meta-analysis.[6] This polymorphism is caused by an amino acid substitution of Gln Q by R at position 192. Its allele frequencies varied among populations.[24] The Q allele is more frequent than R alleles in Caucasians and in Indians. But in Japanese and Chinese, the R allele is more frequent than Q allele. Our results of the allele frequencies are consistent with Chinese living in Singapore[8] and Taiwan.[15] It has been shown that the R allele determines a higher activity of paraoxonase,[25] but in most

studies, it was found to relate to a higher risk of CAD. This may reflect the fact that all the current assays for measurement of enzymatic activity are not indicative of the protective role of *PON1* in preventing LDL oxidation. It has been shown that the PON1 R allozyme is less efficient at retarding the oxidation of LDL than is the Q allozyme because of the decreased hydrolysis of lipid peroxides by the R allozyme.[26] A recent study showed that subjects with 192RR genotype were prone to have coronary spasm,[27] which reflects the endothelium dysfunction related to oxidative damage, providing further supports that the R allele could increase a subject's susceptibility to CAD. However, studies so far have yielded inconsistent findings on the relationship between the *PON1* Q192R polymorphism and CAD. Although these inconsistent findings may simply reflect

Table 4
Joint association of *PON1* Q192R polymorphism and diabetes mellitus (DM) with the risk of MI

| Genotype | DM | No. of Subjects (%) | | Adjusted[a] | | |
		Controls	Cases	OR	95% CI	P
QQ	No	27 (17.5)	20 (13.0)	1.0		
QQ	Yes	4 (2.6)	7 (4.6)	2.3	0.5–10.0	0.27
QR/RR	No	118 (76.6)	94 (61.0)	1.1	0.5–2.3	0.76
QR/RR	Yes	5 (3.2)	33 (21.4)	8.4	2.5–28.0	<0.01

[a]Adjusted OR was calculated by adjusting age, sex, BMI, history of physician diagnosed hypertension and hyperlipidemia, cigarette smoking, and alcohol consumption.

Table 5
Joint association of *PON1* Q192R polymorphism, obesity, and DM with the risk of MI

| Genotype | DM/Obesity status | No. of Subjects (%) | | Adjusted[a] | | |
		Controls	Cases	OR	95% CI	P
QQ	Neither	24 (15.6)	15 (9.7)	1.0		
QQ	Either	6 (3.9)	8 (5.2)	1.9	0.5–7.3	0.34
QQ	Both	1 (0.6)	4 (2.6)	4.5	0.4–55.2	0.24
QR/RR	Neither	101 (65.6)	62 (40.3)	1.1	0.5–2.3	0.89
QR/RR	Either	21 (13.6)	53 (34.4)	3.6	1.5–8.7	<0.01
QR/RR	Both	1 (0.7)	12 (7.8)	13.6	1.5–124.8	0.02

DM/Obesity Status: Neither: the subjects did not have DM nor obesity; Either: the subjects had either DM or obesity; Both: the subjects had both DM and obesity. Obesity is defined as BMI ≥ 27.
[a]Adjusted OR was calculated by adjusting age, sex, history of physician diagnosed hypertension and hyperlipidemia, cigarette smoking, and alcohol consumption.

the imponderables of genetic association studies,[28] another possibility is that, within each study population, individuals carrying the R allele could be at increased risk for CAD only when they are exposed to certain conditions. As *PON1* plays a role in antioxidation, we hypothesize that *PON1* Q192R polymorphism would influence the risk of CAD only in the presence of elevated oxidative stress, for example, among individuals with DM and obesity, two components of metabolic syndrome with high likelihood of insulin resistance. In diabetic subjects, chronic hyperglycemia could cause considerable modification of lipoprotein structure and function due to non-enzymatic glycosylation of amino acid residues and glycosylated LDL is more readily oxidized.[29–31] Insulin resistance could lead to smaller and dense LDL particles, which are more easily oxidized in vitro.[32,33] Two studies demonstrated that insulin resistance is significantly associated with LDL oxidized state in vivo.[34,35] Animal studies as well as in vitro human studies also provided the direct evidence that the oxidative stress increases in obese subjects.[36–38]

Our data demonstrated that the *PON1* Q192R genotype alone in the absence of DM and obesity was not associated with increased risk of MI. In the presence of either DM or obesity, the risk of MI for subjects with QR/RR genotype was considerably increased. The highest risk of MI was found among subjects with QR/RR genotype and with both DM and obesity. Our findings indicate that high oxidative stress may play an important role in modifying the association between the *PON1*

Q192R polymorphism and MI. Our findings underscore the importance to take both DM and obesity into consideration in studying the association between the *PON1* Q192R genotype and the risk of CAD or MI.

Our study represents the very first step in understanding the complex interplay of genetic factors and clinical characteristics in determining individual susceptibility to common disorders such as MI. Results reported in this study are hypothesis-generating only because of the small sample size and single polymorphism that are studied as well as the issue of multiple comparisons. Our findings need to be replicated in other independent populations. If validated, such information could help explain the inconsistent findings on the association between *PON1* Q192R and MI and may help clinicians identify individuals at high-risk for MI.

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