

Original

# The Risk of Coronary Artery Disease in Population of Taiwan Is Associated with Cys-Ser 311 Polymorphism of Human Paraoxonase (PON)-2 Gene

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## Key Words

coronary artery disease;  
high density lipoprotein;  
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polymorphism

**Background.** Paraoxonase (PON), a high density lipoprotein (HDL)-associated enzyme, is capable of inhibiting low density lipoprotein (LDL) oxidation by destroying the biologically active phospholipids in oxidatively modified LDL. An increased risk of coronary artery disease (CAD) has shown to associate with polymorphisms of PON gene (*PON1*) in different population. The risk of CAD associated with the other *PON1*-like gene, designated *PON2*, which has a similar function and is structurally related to *PON1*, is least discussed. A population-based case-control study was conducted to investigate the association between CAD and the polymorphisms at two common codons 148 and 311 of *PON2* in the population of Taiwan. **Methods.** Totally 364 unrelated, angiographically proved CAD-positive patients (338 male and 26 female) and 337 unrelated, CAD-free control subjects (249 male and 88 female) enrolled in this study. Lipids and lipoproteins profile and the association of *PON2* gene types and allele frequencies were analyzed in all study cohorts.

**Results.** The plasma levels of HDL-cholesterol and apoA-I were significantly lower in patients with CAD than in control subjects (both  $p = 0.0001$ ). There was no difference in the genotype frequency distribution at codon 148 of *PON2* between CAD patients and the controls. However, age-, sex- and diabetes-adjusted odds ratios for individuals with the SS genotype of the codon 311 polymorphism (Cys → Ser, *PON2*\*C allele → *PON2*\*S allele) showed a 4.6-fold higher risk of CAD (95% CI = 1.6-15.3,  $p = 0.006$ ) they ran. Also, in the control subjects, *PON2*\*C allele carriers (CC and CS genotypes) had higher plasma levels of HDL than cases with the SS genotypes ( $p = 0.035$  and  $p = 0.012$ , respectively).

**Conclusions.** Our data implicate that the genotypic variation at codon 311 of *PON2* contributes to the susceptibility of CAD in the population of Taiwan. [*Chin Med J (Taipei)* 2002;65:415-421]

It is well documented that oxidized low density lipoprotein (LDL) is involved in the process of atherogenesis.<sup>1</sup> Clinical studies have found that high density lipoprotein (HDL) cholesterol and hypertriglyceridemia have an essential effect on the incidence of coronary artery disease (CAD).<sup>2</sup> *In vitro*

studies have also demonstrated that HDL inhibits the cytotoxicity of oxidized LDL in human cultured endothelial and smooth muscle cells<sup>3</sup> and co-cultures of aortic wall cells.<sup>4</sup> Serum paraoxonase (PON), a HDL-associated enzyme, is capable of inhibiting LDL oxidation by destroying the biologically active

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phospholipids in oxidatively modified LDL.<sup>5</sup> Animal studies have suggested that PON expression may make an important contribution to the ability of HDL to protect against the development of atherosclerotic lesions.<sup>6,7</sup>

Human serum PON, an arylesterase, is a 45 kDa protein and hydrolyzes a broad range of substrates including organophosphates.<sup>8</sup> Serum PON is mainly associated with a subspecies of HDL containing apolipoprotein A-I (apoA-I) and apolipoprotein J (apoJ).<sup>9</sup> The amount of apo A-I-PON-apo J complex increases in the wall of human aorta with the progression of atherosclerosis.<sup>10</sup>

Serum PON activity has been found to be lower in patients with myocardial infarction.<sup>11</sup> There is a wide range of activity and levels of serum PON among human populations of different ethnic backgrounds.<sup>12,13</sup> The human PON gene (called *PON1*) is genetically polymorphic in the human genome. The interindividual variation of PON activity has shown to attribute to the presence of polymorphic variation of a glutamine (*PON1*\*Q or \*A allele) to arginine (*PON1*\*R or \*B allele) at amino acid 192 of the *PON1* gene.<sup>14,15</sup> The R allele of the codon 192 polymorphism of *PON1* has shown to be a genetic risk factor for ischemic heart disease.<sup>16,17</sup> This common polymorphism has also been found to be a determinant of the constrictor effect of human coronary arteries to serotonin.<sup>18</sup> The other polymorphism located at amino acid 55 of the human *PON1*, which involves an interchange of leucine (*PON1*\*L allele) and methionine (*PON1*\*M allele) has been reported to associate with CAD risk among diabetics.<sup>19</sup>

Two additional genes homologous to *PON1*, designated *PON2* and *PON3*, have been identified on human chromosome 7 (7q21-q22). *PON1* has the distinguishing feature of three extra nucleotide residues in exon 4 coding for amino acid 105 compared with *PON2* and *PON3*. It has been suggested that the structurally related *PON2* has a similar function as *PON1* in the metabolism of lipids and lipoproteins.<sup>20,21</sup> The gene variant at codon 148 of *PON2* has shown to worsen glycemia in the native residents of central Canada and Pima Indians with non-insulin dependent diabetics.<sup>22,23</sup> However, very few studies have focused

on the association between *PON2* polymorphism and atherosclerosis. One recent report showed the association of a polymorphism at codon 311 (Cys → Ser; *PON2*\*C allele → *PON2*\*S allele) in the *PON2* with CAD in Asian Indians.<sup>24</sup> We therefore conducted an allelic-association study to investigate the correlation of the polymorphisms at codons 148 and 311 of *PON2* with the risk of CAD in the Chinese population in Taiwan.

## Methods

### Study population

The study subjects, all genetically unrelated Chinese, consisted of 337 control subjects and 364 patients with CAD. Mean age was 61.5 ± 11.8 years for control subjects and 66.9 ± 8.9 years for patients with CAD. The control subjects were healthy volunteers in the Lipid Survey of Veterans General Hospital-Taipei with no history of CAD, with normal electrocardiogram and echocardiography. The patients with CAD were identified from cases admitted to Cardiology Division of Taipei Veterans General Hospital for coronary arteriographic examination. These cases included individuals suffering from angina with the positive treadmill exercise test or Thallium-201 scintigraphic study, and patients scheduled for percutaneous transluminal angioplasty or bypass surgery for documented CAD. In cases with documented myocardial infarction, only those who had had a full recovery for more than 3 months were enrolled. The severity of CAD was determined by fixed stenotic lesion with luminal narrowing ≥ 50% in at least one of the major or minor coronary arteries. Patients with either acute or chronic infectious diseases and malignancy were excluded. All individuals with a fasting serum level of triglycerides higher than 300 mg/dl were excluded.

### Analysis of lipids and lipoproteins

Blood samples mixed with 0.1% ethylenediamine tetraacetic acid (EDTA) were drawn after a 12-hour overnight fasting in all study cohorts. Total cholesterol

terol (TC), the cholesterol content of high density lipoprotein (HDL-C) and low density lipoprotein (LDL-C), and triglyceride (TG) were analyzed enzymatically using commercial reagents (CHOP-PAP Method, Merck Scientific Corporation, Germany). ApoB and apoAI concentrations were determined by a high sensitivity enzyme-linked immunosorbent assay method (AlerCHEK, Inc., Portland, USA).

## Genotyping of DNA

DNA was extracted using standard procedures from leukocytes. A mismatch-PCR assay for genotyping the polymorphism of codon 148 (A [Ala] and G [Gly] alleles) of the *PON2* was carried out as described by Mochizuki *et al.*,<sup>23</sup> and the analysis of the codon 311 polymorphism (S [Ser] and C [Cys] alleles) of *PON2* was carried out as described by Sanghera *et al.*<sup>24</sup> The primer sequences for *PON2* were as follows: 148-sense AGTGGAAAATTTTAAATTTGAAGCAG and 148-antisense TTGTTTGCAAATGCTGGGGAT; 311-sense ACATGCATGTACGGTGGTCTTATA and 311-antisense AGCAATTCATAGATTAATTGTTA. Microplate PCR was conducted with 200  $\mu$ M of each dNTP and 1 U of *Taq* polymerase in a total reaction volume of 20  $\mu$ l. The cycling conditions for the detection of codon 148 polymorphism of *PON2* were 4 min at 94 °C for initial denaturation, followed by 40 cycles of 1 min at 94 °C for denaturation, 1 min 30 sec at 50 °C for annealing and 1 min at 72 °C for extension. The cycling conditions for the detection of codon 311 polymorphisms of *PON2* were 4 min at 94 °C for initial denaturation, followed by 30 cycles of 1 min at 94 °C for denaturation, 1 min 30 sec at 50 °C for annealing and 2 min at 72 °C for extension. All PCR reactions were terminated with a final extension for 7 min at 72 °C. The *PON2* polymorphisms were detected with using restriction enzymes, *Fun4HI* and *DdeI*, respectively. The digested products were separated using 3% MetaPhor agarose gel electrophoresis and visualized using ethidium bromide.

## Statistical analysis

Analysis of the frequency to test for Hardy-

Weinberg equilibrium was performed with  $\chi^2$  goodness-of-fit test. Comparison of the genotype frequencies between CAD cases and controls was calculated by  $\chi^2$  test using a 2  $\times$  3 contingency table. Quantitative variables, including age, body mass index (BMI) and serum lipid levels were compared between the CAD and control groups by the Student's *t* test. Correlation between the serum level of lipid profiles and the genotypes of individual *PON2* genes was examined by ANOVA. Logistic regression analysis was performed to evaluate the interaction between an individual's polymorphism and other variables (such as age and sex) in relation to the prevalence of CAD. A *p* value less than 0.05 was considered significant.

## Results

### Clinical data and lipid profile

The study cohort comprised of 364 genetically unrelated patients with CAD (338 male and 26 female) and 337 control subjects (249 male and 88 female). The clinical data, the plasma levels of the lipid and lipoprotein variables of patients with CAD and control subjects are showed in Table 1. Compared with the control

**Table 1. Clinical data and lipid profile of controls and patients with CAD**

	Controls (n = 337)	CAD patients (n = 364)	<i>p</i> value
Age (years)	61.5 $\pm$ 11.8	66.9 $\pm$ 8.9	0.0001
Sex (M/F)	247/90	338/26	0.001
BMI (kg/m <sup>2</sup> )	24.4 $\pm$ 3.3	24.7 $\pm$ 3.4	0.350
Diabetic (-/+)	327/10	301/63	0.001
Hypertension (-/+)	249/88	189/175	0.001
Smokers (-/+)	207/130	146/218	0.001
Triglyceride (mg/dl)	129.5 $\pm$ 57.5	138.9 $\pm$ 61.3	0.033
TC (mg/dl)	194.6 $\pm$ 50.2	218.6 $\pm$ 65.7	0.0001
HDL-C (mg/dl)	43.6 $\pm$ 14.4	36.6 $\pm$ 12.1	0.0001
LDL-C (mg/dl)	134.0 $\pm$ 50.7	148.0 $\pm$ 63.1	0.0001
ApoA-I (mg/dl)	126.9 $\pm$ 33.3	106.2 $\pm$ 28.4	0.0001
ApoB (mg/dl)	91.4 $\pm$ 26.1	97.5 $\pm$ 34.1	0.0086

CAD = coronary artery disease; TC = total cholesterol; HDL-C = cholesterol content of high density lipoprotein; LDL-C = cholesterol content of low density lipoprotein; ApoA-I = apolipoprotein A-I; ApoB = apolipoprotein B.

group, individuals in the CAD group were older and were more frequently diabetics (17% versus 3%), hypertensive (48% versus 26%) and cigarette smokers (59.9% versus 38.6%). The control group enrolled

more female cases than in the CAD group (26.7% versus 7.1%,  $p = 0.001$ ). Patients with CAD showed significantly higher plasma levels of TG, TC, LDL-C and ApoB than the control subjects ( $p = 0.033$ ,  $p = 0.0001$  and  $p = 0.0086$ , respectively). On the contrary, plasma levels of HDL-C and ApoA-I were significantly lower in patients with CAD than in control subjects (both  $p = 0.0001$ ).

**Table 2. Distribution of *PON2* polymorphisms in controls and patients with CAD**

	Controls	CAD
Codon 148	(n = 328)	(n = 344)
Allele		
A (Ala)	22.6%	18.0%
G (Gly)	77.4%	82.0%
Genotype		
AA	16 (4.9%)	9 (2.6%)
AG	116 (35.4%)	106 (30.8%)
GG	196 (59.7%)	229 (66.6%)
Codon 311	(n = 315)	(n = 364)
Allele:		
S (Ser)	80.5%	84.1%
C (Cys)	19.5%	15.9%
Genotype		
SS	210 (66.7%)	254 (69.8%)
CS	87 (27.6%)	104 (28.6%)
CC <sup>a</sup>	18 (5.7%)	6 (1.6%)

<sup>a</sup>Denotes significant difference between CAD cases and controls,  $\chi^2 = 8.598$  and  $p = 0.017$ .

**Table 3. Calculation of Hardy-Weinberg equilibrium for *PON2* polymorphisms**

	$\chi^2$	<i>p</i> Value
Codon 148		
Controls	0.049	0.976
CAD	0.631	0.730
Codon 311		
Controls	4.627	0.099
CAD	1.615	0.446

### Polymorphisms of *PON2*s and their distribution in patients with CAD and control subjects

The distributions of polymorphisms of *PON2* in the patients with CAD and the control subjects are shown in Table 2. At codon 148 of *PON2*, the distribution pattern of genotype and allele frequency did not differ between patients with CAD and the control subjects. However, at codon 311, the patients with CAD had a significantly higher frequency of the *PON2*\*S allele and a significantly lower frequency of the *PON2*\*C allele (84.1% versus 80.5% and 15.9% versus 19.5%,  $p = 0.014$ ) than the control subjects. The genotype distributions were all in Hardy-Weinberg equilibrium (Table 3). In the *PON2* only model of Table 4, the age-, sex- and diabetes-adjusted odds ratios (ORs) for the development of CAD in the *PON2*\*S carriers (both CS and SS genotypes) was 4.6 (95% CI = 1.6-15.3,  $p = 0.006$ ). In the codominant model in Table 5, the risk of *PON2*\*S allele with separate ORs for CS and SS genotypes gave also significant fits to the data (95% CI = 1.7-16.9,  $p = 0.005$  and 95% CI = 1.6-15.0,  $p = 0.007$ , respectively).

**Table 4. Assessment of risk for development of CAD, calculated by logistic regression**

	OR (95% Confidence Interval); <i>p</i> Value			
	Codon 148 only	Codon 311 only	Codon 148 and Codon 311	Interaction
Age	1.0 (1.0-1.1); 0.0001	1.0 (1.0-1.1); 0.0001	1.0 (1.0-1.1); 0.0001	1.0 (1.0-1.1); 0.0001
Sex	5.2 (3.2-8.9); 0.0001	5.3 (3.2-8.9); 0.0001	5.4 (3.2-9.3); 0.0001	5.3 (3.0-8.9); 0.0001
Diabetes	7.0 (3.4-16.6); 0.0001	8.7 (4.0-21.8); 0.0001	9.6 (4.2-26.1); 0.0001	9.6 (4.2-26.3); 0.0001
Codon 148	1.7 (0.7-4.4); 0.271	---	0.7 (0.2-2.2); 0.552	0.7 (0.1-6.9); 0.724
Codon 311	---	4.6 (1.6-15.3); 0.006 <sup>a</sup>	5.3 (1.7-19.0); 0.006	5.1 (0.7-50.0); 0.122
Codon 148 and Codon 311	---	---	---	1.1 (0.1-13.2); 0.958

Age is tested as continuous variable; Sex is tested with male as the reference group.

<sup>a</sup>Dominant effect test with using CC genotype as the reference group, CC versus CS plus SS.

## Association between lipids profile and genotypes

Genotypes of codon 311 polymorphism of *PON2* on any quantitative traits of lipid and lipoprotein variables are showed in Table 6. Control subjects who were PON\*C carriers (CC and CS genotypes) had a significantly higher plasma level of HDL than cases with the SS genotype ( $p = 0.035$  and  $p = 0.012$ , respectively).

## Discussion

Osei-Hyiaman *et al*<sup>25</sup> reported that *PON1*-192 polymorphism may be an independent risk factor for CAD in the Chinese type 2 diabetes. As found by Sanghera *et al*<sup>24</sup> the *PON1*-192 polymorphism is a sig-

nificant risk factor of CAD in Asia Indians, but not in the Chinese of Singapore. Ko *et al*<sup>26</sup> and our data (not shown) both indicate that codon 192 polymorphism of *PON1* is not associated with CAD in the Chinese subjects in Taiwan.

In the PON gene family, the structurally related *PON2* has a similar function as *PON1* in the metabolism of lipids and lipoproteins. It is accordingly very likely that *PON2* may play an essential role in the susceptibility of CAD. The genetic polymorphism at codon 148 of *PON2* has been reported to be a determinant for fasting hyperglycemia in subjects with type 2 diabetes.<sup>22</sup> Sanghera *et al*<sup>24</sup> indicated that polymorphisms of both codon 192 of *PON1* and codon 311 of *PON2* synergistically contribute to CAD risk in their samples. Our data found that there was no association between the codon 148 polymorphism of *PON2* and CAD in the study subjects. Nevertheless, the codon 311 polymorphism (Cys → Ser) of *PON2* is associated with CAD in the Chinese population of Taiwan. In the present study, PON2\*S allele is a significant risk factor of CAD and is dominant with both the SS and CS genotypes (OR = 4.6) being associated with CAD when compared with the CC genotype. Nevertheless, our data found that diabetes did not affect the association between codon 311 polymorphism of *PON2* and the risk of CAD.

**Table 5. Codominant model of risk of CAD for the S allele at codon 311 of *PON2***

	OR (95% CI)	<i>p</i> value
Sex	5.3 (3.2-9.0)	0.0001
Age	1.0 (1.0-1.1)	0.0001
Diabetes	8.7 (4.0-21.8)	0.0001
Codon 311/CS <sup>a</sup>	5.0 (1.7-16.9)	0.0054
Codon 311/SS <sup>a</sup>	4.5 (1.6-14.9)	0.0074

<sup>a</sup>Denote genotypes of codon 311.

**Table 6. Associations between codon 311 polymorphism of *PON2* and lipid variables in control subjects and patients with CAD**

	Control (n = 315)				CAD (n = 364)			
	CC (n = 18)	CS (n = 87)	SS (n = 210)	CC+CS (n = 105)	CC (n = 6)	CS (n = 104)	SS (n = 254)	CC+CS (n = 109)
Age (years)	60.1 ± 12.4	59.3 ± 12.1	62.2 ± 11.9	59.5 ± 12.1	71.1 ± 6.2	67.1 ± 8.7	66.6 ± 8.6	67.3 ± 8.7
BMI (kg/m <sup>2</sup> )	23.1 ± 3.3	23.7 ± 2.9	24.5 ± 3.4	24.3 ± 3.3	24.0 ± 4.5	24.5 ± 3.3	24.7 ± 3.4	24.5 ± 3.3
TC (mg/dl)	211.9 ± 76.5	233.1 ± 72.4	216.8 ± 63.0	229.5 ± 73.2	206.3 ± 48.3	195.8 ± 54.7	193.2 ± 49.3	196.4 ± 54.2
TG (mg/dl)	120.1 ± 60.6	124.7 ± 58.5	130.8 ± 57.5	123.9 ± 58.6	117.7 ± 40.1	137.6 ± 65.1	138.2 ± 60.0	136.5 ± 64.1
HDL-C (mg/dl)	44.4 ± 19.1	46.6 ± 14.6 <sup>a</sup>	41.9 ± 13.7	46.2 ± 15.4 <sup>b</sup>	38.3 ± 10.5	36.6 ± 13.6	36.5 ± 11.3	36.7 ± 13.4
LDL-C (mg/dl)	146.6 ± 66.8	160.9 ± 66.7	146.6 ± 66.8	158.4 ± 66.7	139.8 ± 41.3	137.0 ± 58.1	131.9 ± 48.2	137.2 ± 57.2
ApoA-I (mg/dl)	130.6 ± 37.7	127.8 ± 33.4	127.5 ± 33.9	128.3 ± 34.0	98.8 ± 32.6	103.3 ± 25.2	107.1 ± 29.5	103.1 ± 25.4
ApoB (mg/dl)	97.9 ± 42.7	101.7 ± 38.4	97.9 ± 42.7	101.1 ± 38.9	99.6 ± 19.7	89.6 ± 24.7	91.9 ± 27.1	90.0 ± 24.5

<sup>a</sup>Denote that the comparison was tested by ANOVA among genotypes,  $p = 0.035$ .

<sup>b</sup>Denote that the comparison was tested for a dominant effect, SS as the reference group, SS versus (CC+CS),  $p = 0.012$ .

CAD = coronary artery disease; TC = total cholesterol; TG = triglyceride; HDL-C = cholesterol content of high density lipoprotein; LDL-C = cholesterol content of low density lipoprotein; ApoA-I = apolipoprotein A-I; ApoB = apolipoprotein B.

The physiological role of association between codon 311 polymorphism of *PON2* and the risk of CAD has not been clarified yet. Plasma PON is mostly bound to large apoA-I in the HDL particle without apoA-II,<sup>27</sup> and the correlation between PON enzyme level and apoA-I and HDL-C has been confirmed.<sup>28</sup> Navin *et al*<sup>29</sup> suggested that the levels of HDL are highly correlated with protein levels of PON, while genotype of codon 192 of *PON1* is the major predictor of the plasma PON activity. Mackness *et al*<sup>30</sup> found that HDL from RR/LL homozygotes of *PON1* was least effective in protecting from the oxidative modification of LDL. Sanghera *et al*<sup>24</sup> has found that the CAD risk as associated with the *PON2*\*S allele was confined to *PON1*\*R allele (R allele of *PON1*-192 polymorphism) carriers. However, their analyses did not discuss the association of polymorphisms of the *PON1* and *PON2* with any trait involving lipid variables. The presence of cysteine at codon 311 in the *PON2* protein is proposed to be responsible for the catalytic activity of *PON2* in dealing with the hydrolysis of oxidized lipid.<sup>31</sup> Therefore, the allelic variation at codon 311 from cysteine to serine may reduce an individual's internal protection against oxidative modification of LDL. Our data showed plasma level of HDL-cholesterol in *PON2*\*C carriers (CC and CS genotypes) higher than in the CAD-free control subjects of the SS genotypes. Since serum PON activity and quantitative data were not available in our analyses, we are unable to confirm the correlation between the plasma PON activity and the genotype of codon 311 of *PON2*. Sanghera *et al*<sup>24</sup> has suggested that both the *PON1*\*R allele and the *PON2*\*S allele merely act as a marker for an unknown functional variant and are in linkage disequilibrium with this functional mutation on one of the PON family genes. In summary, our data indicate that the codon 311 polymorphism of *PON2* is as associated with CAD in the Chinese. However, it is necessary to identify the association between this new mutation in PON gene cluster and the risk of CAD. Further studies on functional aspects of Cys to Ser substitution in lipid peroxidation are necessary in order to explore the correlation between the phenotype of codon 311 polymorphism of *PON2* and the susceptibility to CAD.

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