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Associations between genetic polymorphisms of paraoxonase genes and coronary artery disease in a Taiwanese population

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ABSTRACT

Objective: We evaluated the relationship between polymorphisms of the paraoxonase (*PON*) gene and the risk of coronary artery disease (CAD) in Taiwanese patients.

Methods: Our sample set included 369 volunteers, classified into two groups: 162 healthy volunteers and 207 CAD patients aged 60.0 ± 9.7 and 64.3 ± 12.3 years, respectively. Polymorphisms of the *PON1* and *PON2* genes were determined using polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP) techniques.

Results: The results indicate that for the *PON1* gene, the homozygous genotype RR was found significantly more often among the CAD group compared with the healthy group (OR = 1.965, 95% CI = 1.223–3.159, p = 0.005). Furthermore, for the *PON2* gene, the homozygous genotype CC was found significantly more often among the CAD group compared with the control group (OR = 2.525, 95% CI = 1.103–5.780, p = 0.026).

Conclusions: Individuals homozygous for the R allele of the *PON1* gene and the C allele of the *PON2* gene are more likely to have an increased risk of CAD.

Introduction

A comprehensive survey to determine the major causes of death in Taiwan found that coronary artery disease (CAD) is among the top ten causes year after year, and for many patients, diagnosis may come too late for medical intervention. The *paraoxonase 1* (*PON1*) gene is located on chromosome 7. In humans, *PON1* may play a role in the oxidation of LDL phospholipids [1]. Furthermore, a growing body of evidence indicates that abnormal *PON1* activity may be a risk factor for atherosclerosis-derived CAD. It has been demonstrated that *PON1* knockout mice have a higher rate of atherosclerosis compared with wild-type mice when administered a lipid-rich diet; furthermore, HDL isolated from *PON1* knockout mice has less potent anti-oxidative properties than wild-type HDL [2]. Transgenic mice that express the human *PON1* gene at high levels generate HDL with enhanced anti-oxidative properties, and these mice also have lower rates of atherosclerosis [3]. While these results demonstrate a protective effect for *PON1* against the progression of atherosclerosis, the mechanism through which *PON1* exerts its effects has not yet been defined.

In contrast, the role of paraoxonases related to anti-oxidative and anti-inflammatory processes is more fully understood. The oxidative LDL induced artery wall cells to produce pro-inflammatory molecules. Paraoxonases significantly reduce LDL oxidation in vitro, leading to enhanced HDL-derived protection from inflammatory and vascular sclerosis [4]. Furthermore, compared with a healthy population, serum paraoxonase activity is lower in populations with myocardial infarction, diabetes and familial hypercholesterolemia. Therefore, decreased *PON* activity may be an independent risk factor for cardiovascular disease [4,5].

In recent years, a great amount of effort has been devoted to elucidating the link between *PON1* polymorphisms and cardiovascular disease. The catalytic activity of *PON1* is greatly affected by amino acid substitutions at position 192, and a recent study demonstrated a higher risk of cardiovascular disease for populations carrying the R allele (Q192R) of the *PON1* gene [4,6–8]. Furthermore, the proteins encoded by the *PON1* and *PON2* genes may exhibit overlapping activities. Both *PON1* and *PON2* have been mapped to the short arm of chromosome 7, and due to their proximity, their expression may be regulated in a coordinated manner. For the *PON2* gene, mutations affecting amino-acid position 311 are associated with a higher risk of cardiovascular disease, and it has been demonstrated that the mutation C311S of the *PON2* gene is an independent risk factor for cardiovascular disease in India [6,9].

The aim of this study was to determine the association between *PON1* and *PON2* genetic polymorphisms and CAD in a Taiwanese

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population. These results may be helpful for predicting the development of CAD and for defining appropriate strategies for decreasing mortality through early-stage intervention.

Patients and methods

Sample collection

Sample collections were conducted from April 2009 to February 2011. Three hundred and sixty-nine volunteers were enrolled and classified into two groups. The experimental study group was composed of 207 volunteers (female/male = 40/167) with an age range of 31–93 years (median = 60). All patients were admitted to the Cardiology Department at Landseed Hospital and were examined by a coronary angiogram. CAD patients were defined as a hypoxia report in treadmill exercise test and a thrombosis affecting greater than 50% of the area occurring in at least one of the coronary arteries, as determined by an angiogram. One hundred and sixty two volunteers were selected as a control group (female/male = 71/91) with an age range of 27–80 years (median = 59). Individuals were defined as normal when they showed no clinical symptoms in regular physical check followed by treadmill exercise test. All participants were clearly informed about the potential risks of the study and voluntarily signed the agreement with IRB approval.

DNA extraction

Sample DNA was extracted using the EasyPure Genomic DNA spin kit (BIOMAN SCIENTIFIC CO., LTD, Taiwan) from 2–3 mL of ETDA-treated blood. The extraction processes were carried out according to the manufacturer's recommendations, and the resultant DNA was re-suspended in sterile water. The quality of the DNA samples was determined using a spectrophotometer (WPA BIOWAVE II), and $OD_{A260/280}$ values from 1.8 to 2.0 were observed. All DNA preparations were stored at −20 °C prior to use.

Genotyping

PCR–RFLP techniques were used to determine the *PON1* and *PON2* genotypes as previously described [9]. For the PCR amplifications, 100 ng of genomic DNA was used as template, and the other reagents were assembled according to the supplier's protocol (BIOMAN SCIENTIFIC CO., LTD, Taiwan). The primers used are listed below:*PON1* forward: 5′-TATTGTTGCTGTGGGACCTGAG-3′; and *PON1* reverse: 5′-CACGCTAAACCCAAATACATCTC-3′. The PCRs (*PON1*-Q192R) were performed in a total volume of 40 μL containing: 1 U *Taq* polymerase, 2.5 mM $MgCl_2$ *Taq* buffer, 1 mM dNTP (deoxynucleotide triphosphate), 0.5 μM primers, and 100 ng DNA. The reaction was performed under the following conditions: an initial denaturation at 94 °C for 5 min, followed by 30 cycles of denaturation at 94 °C for 30 s, annealing at 61 °C for 30 s, elongation at 72 °C for 60 s, and final extension at 72 °C for 5 min. The amplified DNA fragments with 99 bp in size were cleaved with 4 U *Alw*I (BIOMAN SCIENTIFIC CO., LTD, Taiwan) at 55 °C for 8 h. After digestion, the following patterns were obtained: one fragment of 99 bp for homozygous genotype QQ, 3 fragments of 99 bp, 63 bp and 36 bp for heterozygous genotype QR, and 2 fragments of 63 bp and 36 bp for homozygous genotype RR. The primers for *PON2* gene amplification were *PON2* forward: 5′-ACATGCATGTACGGTGGTC TTATA-3′ and *PON2* reverse: 5′-AGCAATTCATAGATTAATTGTTA-3′. Similarly, after the DNA was denatured at 94 °C for 5 min, the reaction mixture was subjected to 30 cycles of denaturation at 94 °C for 30 s, annealing at 45 °C for 30 s, and extension at 72 °C for 1 min. The amplified products with 262 bp in size were cleaved with 4 U *Dde*I (BIOMAN SCIENTIFIC CO., LTD, Taiwan) at 37 °C for 8 h. After digestion, the following patterns were obtained: 2 fragments of 142 bp and 120 bp for homozygous genotype CC, 4 fragments of 142 bp, 120 bp, 75 bp and 67 bp for heterozygous genotype CS, and 3 fragments of 120 bp, 75 bp and 67 bp for homozygous genotype SS. The resultant DNA fragments were analyzed using 4% agarose gel electrophoresis.

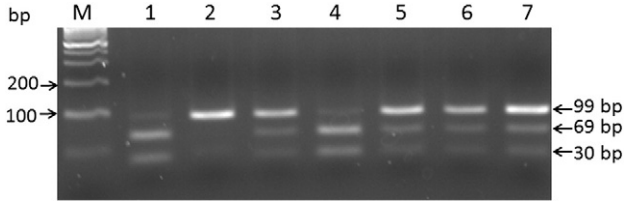

Fig. 1. *PON1* genotyping by PCR–RFLP. PCR products were treated with *Alw*I restriction enzyme and subjected to electrophoresis on a 4% agarose gel. Lane M: 100 bp DNA ladder; lane 2: a homozygous QQ genotype; lanes 1 and 4: a homozygous RR genotype; lanes 3, 4 and 6: a heterozygous QR genotype; and lane 7: a positive control.

Statistical analysis

Statistical analyses were performed using the Statistical Package for Social Sciences (SPSS Inc., Chicago). Allelic frequencies and genotypic distributions were analyzed by Chi-square test. The odds ratios used for determining the association between risk factors and CAD were calculated using multivariate logistic regression.

Results

The presence of allele Q at the *PON1* locus was indicated by a 99 bp fragment, as visualized using gel electrophoresis (Fig. 1). Additionally, the presence of allele R at the *PON1* locus was indicated by a pair of 69 and 30 bp fragments, as digested by *Alw*I. Genotyping of the *PON2* locus is described in Fig. 2. The presence of allele C at the *PON2* locus was indicated by a pair of 142 and 120 bp fragments. A triplet composed of 120, 75 and 67 bp fragments indicated the presence of allele S at the *PON2* locus.

The demographic characteristics of the tested subjects, including mean age (control group, 60 vs. CAD group, 64.3), female/male gender ratio (control group, 71/91 vs. CAD group, 40/167), hypertension (control group, 61.0% vs. CAD group, 52.7%), hypercholesterolemia (control group, 57.4% vs. CAD group, 50.2%) and diabetes (control group, 32.7% vs. CAD group, 28.0%), were analyzed using statistical measurements, and the results are shown in Table 1. Smaller p values indicate that *PON1* and *PON2* genetic polymorphisms are more significantly associated with the CAD group than with the control group.

Our data (Tables 2 and 3) indicate that the frequencies of the *PON1* genetic subtypes in the Taiwanese CAD population are 0.51 for allele R and 0.49 for allele Q. It is intriguing that for the *PON1* gene, the homozygous genotype RR of is found significantly more often in the CAD group than in the control group (OR = 1.965, 95% CI = 1.223–3.159, p = 0.005). For the *PON2* gene, the distribution frequencies for alleles C and S are 0.23 and 0.77, respectively. Furthermore, for the *PON2* gene, the homozygous genotype CC is found significantly more often in the CAD group than in the control group (OR = 2.525, 95% CI = 1.103–5.780, p = 0.026).

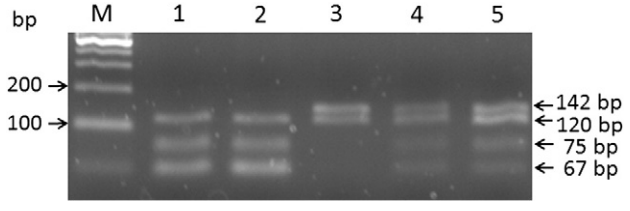

Fig. 2. *PON2* genotyping by PCR–RFLP. PCR products were treated with DedI restriction enzyme and subjected to electrophoresis on a 4% agarose gel. Lane M: 100 bp DNA ladder; lanes 1 and 2: a homozygous SS genotype; lane 3: a homozygous CC genotype; lane 4: a heterozygous CS genotype; and lane 5: a positive control.

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