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Original Article

Relationship between *GPX1* rs1050450 variation and the onset risk of coronary artery disease

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Abstract: Objective: In order to explore the relationship between glutathine peroxidase (*GPX1*) rs1050450 polymorphism and the onset of coronary artery disease (CAD) in a Han population of China, we designed this case-control study. Methods: We invited 103 CAD patients and 112 healthy persons to participate in this study. The CAD patients were defined as patients with at least one coronary artery stenosis of ≥50% luminal diameter. Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method was adopted for genotyping the *GPX1* polymorphisms. The frequencies of genotypes and alleles, and the association of polymorphisms with CAD susceptibility were analyzed by χ^2 test. Results: In rs1050450 polymorphism, the frequency distribution differences of mutant genotype CC+CT in case and control groups were statistically significant, and CC+CT could increase 2.24 times the onset risk of CAD compared with CC genotype (OR=2.24, 95% CI=1.09-4.60). Meanwhile, T allele was found to also can increase the onset risk of CAD. Further correlation analysis of *GPX1* rs1050450 polymorphism and the severity of coronary lesion found that the distribution differences of CT and TT genotypes and T allele among single vessel lesion, double vessel lesion and three vessel lesion had no statistical significance (P>0.05). Conclusions: *GPX1* rs1050450 polymorphism is associated with the onset of CAD, but not with the severity of coronary lesion.

Keywords: *GPX1*, CAD, variation

Introduction

Coronary artery disease (CAD) patients are patients with atherosclerotic lesion, and here follows the formation process of such lesion: the abnormal lipid metabolism makes the lipids in blood adhere to the smooth endarterium, and some atheromatous lipids on the endarterium then pile up to form white patches, and these patches are called atherosclerotic lesion [1]. The increasing patches stenose the lumen artery, block the blood flow and finally lead to cardiac ischemia and angor pectoris. The infected people are mainly middle-to-old-aged population. Males over 45 years and females over the age of 55 or in the post-menopause stage are the high risk populations. Moreover, there exist other risk factors such as genetic family history, hypertension, diabetes, smoking, overweight, obesity, gout and lack of exercises. In recent years, with the change of life style, the improvement of living standard and the variation of environmental factors, the incidence of CAD has presented a clear ascendant trend [2, 3]. At present, scientists generally believe that the occurrence of CAD is affected by the combination of genetic factors and environmental factors, and several environmental factors can affect the heredity susceptibility to CAD [4]. However, researches have shown that traditional risk factors only account for 50% of the pathogenesis of CAD [5]. Lusis et al. pointed out that in the causes of CAD, the effects of genetic factors accounted for about 40% to 60%, and its influences on premature CAD were greater than on late-onset CAD [6, 7]. Thus it can be concluded that the onset of CAD has obvious genetic predisposition and familial aggregation.

Oxidative stress (OS) is considered by related studies to be the central link of atherosclerosis that plays an important role in the occurrence and development process of CAD [8-11]. Glutathine peroxidase (*GPX1*) is a kind of selenium protease with an antioxidant effect. It mainly expresses in the epidermal tissues of the body, and is an important component of the

Table 1. Basic information comparison between cases and controls

Information	Case (n=103)	Control (n=112)	*P*
Age	56.48±6.5	58.28±10.6	*P*>0.05
Gender (male/female)	57/46	61/51	*P*>0.05
Smoking	59 (57)	39 (34)	*P*<0.05
Body mass index (kg/m^2)	25.14±3.13	24.26±3.25	*P*>0.05
Systolic blood pressure (mmHg)	132.8±24.6	116.2±16.7	*P*<0.05
Diastolic blood pressure (mmHg)	79.1±14.3	83.26±12.3	P>0.05
Hypertension	62 (60)	35 (31)	P<0.05
Diabetes	25 (24)	13 (11)	P<0.05

Table 2. Genotype and allele distributions comparison of *GPX1* rs1050450 polymorphism in two groups

Group	Case load	Genotype				Case load	Allele	
		CC	CT	TT	CT+TT		C	T
Case	103	78	20	5	25	206	176	30
Control	112	98	13	1	14	224	209	15
P		0.03					0.01	
χ^2		5.01					7.09	
OR (95% CI)		2.224 (1.09-4.60)					2.38 (1.24-4.56)	

Table 3. Genotype and allele distributions of *GPX1* in single vessel lesion, double vessel lesion and three vessel lesion

Group	Case load	Genotype		Case load	Allele	
		CC	CT+TT		C	T
Single vessel lesion	44	33	11	44	74	14
Double vessel lesion	39	30	9	39	68	10
Three vessel lesion	20	15	5	20	34	6
P		*P*>0.05			*P*>0.05	

antioxidant enzymes defense system [12]. Many studies suggested that *GPX1* played a vital part in protecting the vessel wall for antioxidative stress and against atherosclerosis [13-15]. Related researches on the correlation between *GPX1* rs1050450 polymorphism and CAD are rare and the results are controversial. Therefore, we applied polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method to analyze *GPX1* rs1050450 polymorphism and discuss its association with CAD susceptibility in Chinese Han population.

Materials and methods

Description of the study subjects

103 CAD patients (57 males and 46 females) aged 56.48±6.5 years and treated in affiliated hospital of Hebei Engineering University was recruited into the case group. The cases were diagnosed by symptoms of more than one coronary artery with 50% or higher diameter stenosis, and their clinical diagnoses were in accordance with the diagnostic criteria published by ISFC/WHO. 112 homochronous healthy persons (61 males and 51 females) that took physical examination at the same hospital were randomly recruited into the control group. The controls with an average age of 58.28±10.6 were examined by electrocardiogram (ECG) and laboratory examinations without chest pain. The unrelated cases and controls were all Han population of China. They did not have diabetes, rheumatic heart disease or severe hepatic insufficiency. The participants were fully informed and they agreed to sign informed consent. The study was conducted according to the guidelines of the ethnic committee of affiliated hospital of Hebei Engineering University.

Sample extraction

5 ml antecubital venous blood samples were drawn in the morning after an overnight fasting period and then put into EDTA anticoagulative tubes. Then we collected genome DNA from the blood sample with genomic DNA extraction kits from TAKARA Company.

PCR reaction for genotype determination

We adopted PCR-RFLP method for *GPX1* rs1050450 polymorphism detection. The forward primer sequence was 5'-CGCTTCCAGACCATT GACATC-3', and the reverse primer sequence was 5'-GGACCAGCACCCATCTCGA-3'. With a total of 25 μL, the PCR reaction system consisted of genome DNA 100 ng, PCR Master Mix (MBI Fermentas, Lithuania) 12.5 μL, H_2O 10 μL, and forward and reverse primers 0.2 μL

each. The reaction conditions were: 5 min warm start at 94°C; 35 cycles of 94°C for 30 s, 60°C for 30 s, 72°C for 30 s; at last extension at 72°C for 10 min. 10 μL amplification products were mixed with 6 U Apa I restriction enzyme (GGGCCC, MBI Fermentas, Lithuania) and the mixture was incubated in a water bath at 37°C for 3 h. Then we performed 2% agarose gel electrophoresis and determined the genotypes under UV transilluminators. The determination results were presented as: CC genotype with two stripes of 156 bp and 57 bp, CT genotype with three stripes of 213 bp, 156 bp and 57 bp, TT genotype with a 213 bp stripe.

Statistical analysis

SPSS 18.0 software was applied to perform statistical processing. Differences between groups were compared by χ^2 test. Hardy-Weinberg equilibrium (HWE) was used to check whether the genotype distribution frequencies of *GPX1* polymorphisms conform to group representativeness. The effects of *GPX1* polymorphisms on CAD were represented by odds ratios (OR) and 95% confidence intervals (CI). When $P<0.05$, the indicators were considered with statistical significance.

Results

Basic information comparison between case and control groups

The comparison of the general features between case and control groups is given in **Table 1**. It seemed that the two groups had same base-line conditions, and the differences in age, sex, body mass index (BMI) and diastolic blood pressure (DBP) had no statistical significance. However, the frequency distributions of smokers, diabetes patients and hypertension patients, as well as the level of systolic blood pressure (SBP) were higher in case group than in control group, and the differences were significant (*P* values less than 0.05).

Frequency analyses of the genotypes and alleles in GPX1 rs1050450 polymorphism

The genotype distributions of rs1050450 polymorphism in control group were in HWE ($\chi^2=0.57$, $P=0.45$). The genotype and allele distribution frequencies of *GPX1* rs1050450 polymorphism can be found in **Table 2**. From the table we could see that there existed CC, CT and TT three genotypes in cases and controls. The distribution frequencies of TT genotype were low in two groups, so we combined two mutant genotypes of CT and TT to detect the frequencies and it turned out that CT+TT was more frequent in case group than in control group and the difference was statistically significant ($P<0.05$). The risk of people with these two genotypes developing CAD was increased by 2.24 times (OR=2.24, 95% CI=1.09-4.60), and it indicated that the mutant genotypes of *GPX1* rs1050450 polymorphism might be risk factors of CAD. In the mean time, the distributions of T allele were found significantly different in two groups ($P=0.01$), and T allele could remarkably increase the risk of developing CAD when compared with C allele (OR=2.38, 95% CI=1.24-4.56). It suggested that the mutant genotypes of *GPX1* rs1050450 polymorphism correlated with the onset of CAD.

*Relationship between GPX1 rs1050450 polymorphism and the severity of CAD (**Table 3**)*

Further analyses made us conclude that the distributions of CT+TT genotype and T allele in single vessel lesion, double vessel lesion and three vessel lesion had no statistical significance of comparable differences ($P>0.05$), and it manifested that *GPX1* rs1050450 polymorphism had nothing to do with the severity of CAD.

Discussion

In recent years, cardiovascular diseases (CVDs) have topped the causes of death in human beings. As a common chronic CVD, CAD has caused serious harm to human health and quality of life. With the increase of CAD incidence, it has become one of the main diseases leading to death and disability both in and out of China. Therefore, continuous exploration on the pathogenic mechanism and related risk factors of CAD has important theoretical significance and application value for the prevention and treatment of the disease.

With the deepening of the research on the pathogenesis of CAD, the roles of *GPX1* played in the onset of CAD have drawn more and more attentions. There are plenty of studies which show that *GPX1* plays a great role in protecting for anti-oxidative stress and against athero-

sclerosis of the vessel wall [16-19]. GPX is a kind of selenium protein and an important component of antioxidant enzymes system. It can protect the DNA protein and lipids from the damages caused by hydrogen peroxide and lipid peroxidation. At least six GPX isoenzymes so far discovered all have selenocysteine in their active sites [20]. *GPX1* is an important component of the glutathione peroxidase family. It widely distributes in the body tissues, and can catalyze and decompose the hydrogen peroxide and lipid peroxide into water and corresponding aldehyde [21-23]. Studies have found that *GPX1* 200Leu mutant enzyme has lower activity than 200Pro wild enzyme [24]. Thus it may increase the produce of OS and further increase the risk of CAD.

As for the association of *GPX1* polymorphisms with CAD, Tang et al. ascertained through their studies that *GPX1* rs1050450 polymorphism was related to CAD in Chinese populations [25]. Sergeeva et al. illustrated that *GPX1* Pro198Leu polymorphism had no significant relationship with myocardial infarction and shock in hypertension patients along with non-insulin-dependent diabetes mellitus in Russia [26]. The present study, however, demonstrated that *GPX1* rs1050450 polymorphism was associated with the genetic susceptibility to CAD. The mutant genotype CT+TT of rs1050450 polymorphism had a higher frequency in case group than in control group, and the difference was statistically significant, which indicated that genotype CT+TT was a risk factor of CAD. Similarly, T allele could also increase the risk of developing CAD and it correlated with the onset of the disease. But *GPX1* rs1050450 polymorphism corresponded not to the severity of coronary lesions.

The research results on the association of *GPX1* rs1050450 polymorphism with CAD are not consistent till now and there still exist some big differences. CAD is a multifactorial inheritance disease with a complex pathogenesis. It is the result of the interaction between environmental factors and genetic factors, and its genotypes distributions are different because of the different race and region. Besides, the sample size we collected is relatively small, thus the results may have differences. Therefore, study on the correlation between *GPX1* rs1050450 polymorphism and CAD needs to be further discussed and confirmed with a larger sample size.

Disclosure of conflict of interest

None.

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