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Original Article

PPARG, *AGTR1*, *CXCL16* and *LGALS2* polymorphisms are correlated with the risk for coronary heart disease

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Abstract: Purpose: Our study was designed to explore the interaction between genes of *PPARG*, *AGTR1*, *CXCL16* and *LGALS2* and further investigate the association between genes polymorphisms and coronary heart disease (CHD). Methods: 90 CHD patients and 80 healthy individuals were enrolled in our study. Gene chip technology was used for checking four single nucleotide polymorphisms (SNPs) (*PPARG* rs1152002, *AGTR1* rs5186, *CXCL16* rs3744700 and *LGALS2* rs7291467). MDR software was used to analyze gene-gene interactions. Odds ratio (OR) with 95% confidence interval (CI) were employed to evaluate the association of genes and CHD risk. Results: Genotypes and alleles distribution in case and control groups showed significant difference (P<0.05). And there exists interaction among genes. The model of *PPARG*×*CXCL16* showed effects on the occurrence of CHD (OR=2.92, 95% CI=1.44-5.94). Meanwhile, the *PPARG*×*AGTR1*×*CXCL16*×*LGALS2* model was associated with CHD susceptibility (OR=3.97, 95% CI=2.01-7.84). Moreover, we found that *PPARG*×*LGALS2*×*CXCL16*, was the best interaction model and it could significantly increase the risk for CHD (OR=3.37, 95% CI=1.71-6.63). Conclusion: *PPARG* rs1152002, *AGTR1* rs5186, *CXCL16* rs3744700 and *LGALS2* rs7291467 polymorphisms may be closely related to the development of CHD. Moreover, there exist gene-gene interactions among these susceptibility genes.

Keywords: Coronary heart disease, *PPARG, AGTR1, CXCL16, LGALS2,* polymorphisms

Introduction

Coronary heart disease (CHD) including myocardial infarction (MI) is a common disease leading to death and disability all over the world [1]. CHD is the leading cause of death in developed countries, and its incidence is rapidly increasing in developing countries. Both environmental and genetic factors play non-negligible roles in the occurrence and development of CHD [2-4].

PPAR was first found and named by Issemann *et al*., in 1990 [5, 6], which is a member of the nuclear receptor superfamily. According to structural characteristics, *PPAR* can be divided into α, β and γ types. *PPARγ* (*PPARG*), a crucial factor in differentiation of preadipocyte and a target molecule of thiazolidinediones, which mainly expresses in adipose tissues and immune system and is closely related to adipocyte differentiation, body immunity and insulin resistance [7]. Moreover, it has been demonstrated that *PPARG* is related to various diseases such as diabetes, obesity and atherosclerosis [8-10]. As an important member of renin-angiotensin system, angiotension II type 1 receptor (*AGTR1*) is also associated with high risk for CHD [11]. *CXCL16* is a recently discovered tranmembrane chemoking and Huang et al. has reported that it plays crucial role in the pathogenesis of CHD [12]. In addition, Lian et al. found that lectin galactoside-binding soluble-2 (*LGALS2*) appeared to influence the progress of CHD [13].

In recent years, studies have found that single nucleotide polymorphisms (SNPs) are correlated with CHD susceptibility, whereas most of them are only confined to individual SNPs. Considering rare research on gene-gene interactions involved in the pathogenesis of CHD, we

Table 1. Test results of *PPARG*, *AGTR1*, *CXCL16* and *LGALS2* polymorphisms

SNP	Test result
PPARG rs1152002	+ (AA)/- (GG)/± (GA)
AGTR1 rs5186	+ (CC)/- (AA)/± (AC)
CXCL16 rs3744700	+ (TT)/- (GG)/± (GT)
LGALS2 rs7291467	+ (AA)/- (GG)/± (GA)

+: homozygote harmful to health; ±: heterozygote; -: homozygote beneficial to health.

Table 2. Comparison of clinical materials

Item	Case	Control
Hypertension	56	36
Diabetes	22	18
Smoking	54	37
Drinking	38	20
Age	62.40±11.71	65.34±12.47
Gender (Male/Female)	64/26	57/23
BMI (kg/m^2)	24.77±2.89	24.96±3.32
TC (mmol/L)	4.77±1.14	4.82±1.28
TG (mmol/L)	2.08±1.56	1.86±0.92
HDL (mmol/L)	1.08±0.29	1.14±0.31
LVDd (mm)	52.86±8.37	51.28±9.63
LVSd (mm)	34.27±11.28	35.28±12.31
EF (%)	54.74±12.68	58.73±13.96

explored the interaction between above genes and studied the relationship of polymorphisms of the genes (*PPARG* rs1152002, *AGTR1* rs5186, *CXCL16* rs3744700, *LGALS2* rs7291467) with CHD risk.

Materials

All the participants were selected from Department of Cardiology in Xijing Hospital from February 2012 to August 2014, which were diagnosed by two experienced physicians through the examinations of electrocardiogram, myocardial enzyme, heart color ultrasound and angiography. The participants with no genetic connection in Chinese Hans were divided into two groups (case and control group). In the case group, 90 patients included 64 males and 26 females with mean age of 62.4±11.71. The subjects were verified to be at least one coronary artery with stenoses (≥50%) among three primary blood arteries (right coronary artery, anterior descending branch and circumflex branch) or two left main coronary arteries by coronary arteriongraphy. In the control group, 80 individuals including 57 men and 23 women (mean age: 65.34±12.47) only had atypical chest pains. Those with infectious diseases, rheumatic diseases and tumors, coronary artery stenosis smaller than 50%, and apparently abnormal hepatic and renal functions were precluded from the study.

Methods

Collection of clinical materials

Fasting venous blood was extracted from the patients and then centrifugated. TC, TG, HDL-C and LDL-C were tested with the method of double reagent enzyme. Left ventricular diastolic diameter (LVDd) and left ventricular systolic diameter (LVSd) were two-dimensionally measured using Hp5500 the ultrasonocardiograph. Left ventricular ejection fraction (EF) was calculated based on the formula of EF=(LVDd-LVSd)/LVDd.

Detection of SNP

Two ml of peripheral blood was collected and anticoagulated with EDTA-2Na. Genomic DNA was extracted according to instructions of the extraction kit and then was stored at -20°C for further detection. *PPARG* rs1152002 (GG, GA, AA), *AGTR1* rs5186 (AA, AC, CC), *CXCL16* rs3744700 (GG, GT, TT) and *LGALS2* rs7291467 (GG, GA, AA) polymorphisms were all tested by gene chip technology (Shanghai BaiO Technology Co., Ltd). The main instruments used in the study were as follows: centrifuge (TGL-16G, Shanghai Jiapeng Technology Co., Ltd); nucleic acid protein detector (Shanghai Shsk Industrial Co., Ltd); GeneAmp PCR system (9700, ABI, US); gel rapid documentation apparatus (JUNYI ELECTROPHORESIS); biochip reader (Shanghai BaiO Technology Co., Ltd) and Array Doctor 2.0 (Shanghai BaiO Technology Co., Ltd). The detection results would be automatically output if the comparison of negative and positive samples was qualified with software. Test results were showed in **Table 1**.

Statistical methods

Measurement data were presented as $\bar{X} \pm s$. T test was used for the comparison of data in different groups. And X^2 test was applied to compare distributional differences of each SNP between case and control group. Gene interaction model was determined using MDR1.10

Table 3. HWE test

SNP	Frequency of case	*P*	Frequency of control	*P*
rs1152002	0.4820	0.9123	0.4303	0.2998
rs5186	0.0473	0.2685	0.0291	0.5038
rs3744700	0.0914	0.6491	0.1308	0.4654
rs7291467	0.2774	0.1868	0.3343	0.5505

Table 4. Distributional frequency of genotypes and alleles (%)

Genotype	Case (n=90)	Control (n=80)	*P*	Allele	Case (2n=180)	Control (2n=160)	P
rs1152002							
GG	12 (13.3)	19 (23.7)	0.046	G	72 (0.40)	78 (48.8)	0.035
GA	48 (53.3)	40 (50.0)					
AA	30 (33.3)	21 (26.3)		A	108 (0.60)	82 (51.2)	
rs5186							
AA	86 (95.6)	68 (85.0)	0.033	A	176 (97.8)	148 (92.5)	0.037
AC	4 (4.4)	12 (15.0)					
CC	0 (0.0)	0 (0.0)		C	4 (2.2)	12 (7.5)	
rs3744700							
GG	62 (68.9)	66 (82.5)	0.039	G	149 (82.8)	145 (90.6)	0.039
GT	25 (27.8)	13 (16.3)					
TT	3 (3.3)	1 (1.2)		T	31 (17.2)	15 (9.4)	
rs7291467							
GG	35 (38.9)	44 (55.0)	0.040	G	114 (63.3)	118 (73.8)	0.047
GA	44 (48.9)	30 (37.5)					
AA	11 (12.2)	6 (7.5)		A	66 (36.7)	42 (26.2)	

Table 5. Interaction models and performance evaluations

Interaction model	Training sample accuracy	Checking sample accuracy	Cross validation consistency	χ^2 (*P*)	OR (95% CI)
PPARG×CXCL16	0.6132	0.6096	10/10	9.08 (0.0026)	2.92 (1.44-5.94)
PPARG×LGALS2×CXCL16	0.6417	0.6327	10/10	12.79 (0.0003)	3.37 (1.71-6.63)
PPARG×AGTR1×CXCL16×LGALS2	0.6620	0.5881	10/10	16.44 (<0.0001)	3.97 (2.01-7.84)

according to the standards of largest cross validation consistency coefficient and highest checking sample accuracy. There is statistical significance when *P* value is smaller than 0.05. All data analyses were conducted in SPSS 18.0 software.

Results

Comparison of clinical data

The differences on clinical data (hypertension, diabetes, smoking, drinking, age, gender, BMI, TC, TG, HDL, LVDd, LVSd and EF) between case and control group were analyzed. As shown in **Table 2**, no significant differences were found (*P*>0.05), which suggested that clinical factors of hypertension, diabetes, smoking, drinking, age, gender, BMI, TC, TG, HDL, LVDd, LVSd and EF were not associated with CHD susceptibility.

HWE examination

As demonstrated in **Table 3**, genotypes distribution of the four SNPs were accorded with HWE (*P*>0.05), suggesting that all samples were representative.

Comparison of genotype and allele frequencies

As shown in **Table 4**, compared with the control group, we found noteworthy statistical differ-

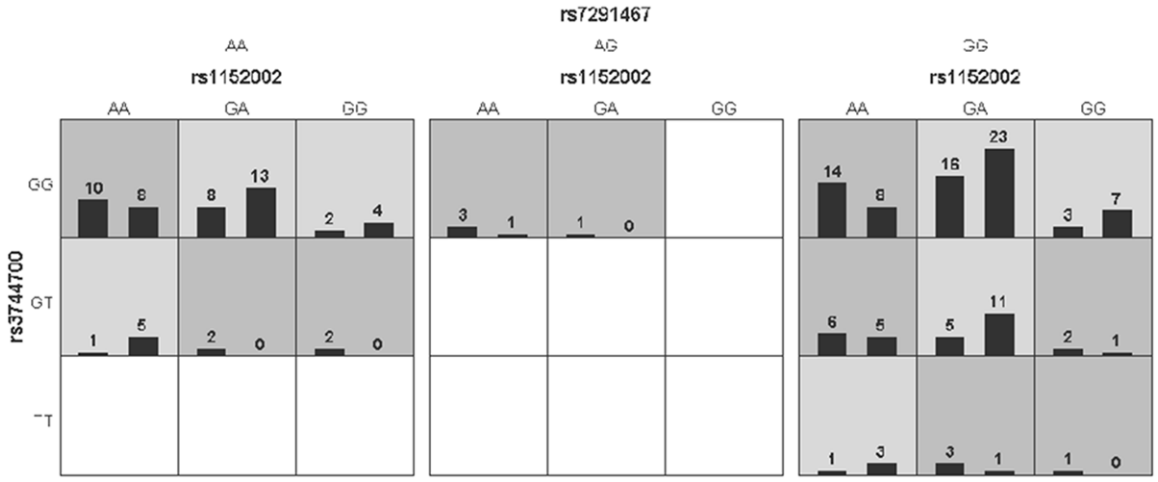

Figure 1. Genotype combination of *PPARG×LGALS2×CXCL16* interaction model. The left band stands for case group and right band for control group. Dark grey color indicates high risk (ratio of number of cases and controls ≥1), light grey color indicates low risk (ratio of number of cases and controls <1), white color represents no data.

Table 6. Risk level of genotype combination of *PPARG×LGALS2×CXCL16* interaction model

	PPARG rs1152002G/A	*LGALS2* rs7291467G/A	*CXCL16* rs3744700G/T
High risk	AA	AA	GG
	GA	AA	GT
	GG	AA	GT
	AA	AG	GG
	GA	AG	GG
	AA	GG	GG
	AA	GG	GT
	GA	GG	TT
	GG	GG	GT
	GG	GG	TT
Low risk	GA	AA	GG
	GG	AA	GG
	AA	AA	GT
	GA	GG	GG
	GG	GG	GG
	GA	GG	GT
	AA	GG	TT
No data	-	-	-

ences of genotype and allele frequencies in case group (P<0.05).

Analysis of gene-gene interactions

With MDR software, we analyzed gene-gene interactions of the four SNPs and obtained three interaction models (*PPARG×CXCL16*, *PPARG×LGALS2×CXCL16*, *PPARG×AGTR1×CXCL16×LGALS2*), which presented statistical significance through permutation test (P<0.05). *PPARG×LGALS2×CXCL16* model was further identified as the best one for its higher cross validation consistency coefficient (10/10) and checking sample accuracy (0.6327) (**Table 5**). And further analysis indicated that the three models were all significantly correlated with the risk of CHD (OR=2.92 95% CI=1.44-5.94; OR=3.37 95% CI=1.71-6.63; OR=3.97, 95% CI=2.01-7.84) (**Table 5**). The risk estimate of *PPARG×LGALS2×CXCL16* interaction model was showed in **Figure 1** and **Table 6**.

Discussion

According to the estimates of world health organization (WHO), cardiovascular diseases will emerge as the main cause of human death by the year of 2020 [14, 15]. The global prevalence of the complicated diseases and corresponding spiral rise of hospitalization costs bring huge burden to countries especially developing countries in the world [16, 17]. Therefore, radically figuring out the pathogenesis of the diseases is crucial for prevention and diagnosis of diseases, drug design and personalized treatment.

It is widely believed in recent studies that cardiovascular diseases including CHD are caused by combined actions of SNPs and environmental factors, and that different combinations of

SNPs confer different susceptibility to cardiovascular diseases, thus leading to different clinical features and therapeutic effects of corresponding diseases [18, 19]. As a result, study on candidate SNPs in relation to cardiovascular diseases has attracted extensive attention [20]. New strategy studies on susceptibility genes in relation to hereditary diseases covering systemic sclerosis and rheumatoid arthritis suggest that the positive result is rare in a single SNP. The occurrence of diseases are commonly caused by SNPs in various genes [21].

Taking South Asians, Chinese and European Caucasians as subjects and testing 321 SNPs of 15 genes, Lanktree M *et al*., suggested that *PPARG* polymorphisms were related to IMT ($P<0.05$), the results of which were adjusted by the factors of race, age, gender, systolic pressure, BMI, smoking and lipid-lowering treatment [22]. Huang *et al*., selected 1176 CHD patients and 850 controls in Chinese Han population and investigated the function of four SNPs of *CXCL16* in the pathogenesis of CHD. Consequently, genotypes and alleles of rs3744700 were all related to CHD ($P=0.001$, $P<0.001$) [23]. In a study conducted by Abdollahi *et al*., CC genotype of *AGTR1* rs5186 was found in relation to the risk of cardiovascular diseases [24]. A large number of studies have indicated that inflammatory response plays an important part in occurrence of atherosclerosis and cerebrovascular diseases. In some investigations, atherosclerosis is even considered as an inflammatory disease and inflammatory response of atheromatous plaques is the main cause of corrosion and rupture of arterial walls. Galectin-2 is able to regulate the intracellular transportation of a proinflammatory factor called lymphotoxin-α (LTA). The level of galectin-2 is determined by *LGALS2* gene which can directly control cytokinin levels of LTA. In three SNPs of *LGALS2* gene, rs7291467 was reported to be a susceptibility polymorphism to arteriosclerosis and cardiovascular and cerebrovascular diseases [25, 26].

In the present study, we selected above four SNPs associated with CHD and intima media thickness (IMT) with 90 CHD cases and 80 healthy controls to analyze the association of SNPs with CHD. Our study suggested that rs1152002 of *PPARG* was associated with CHD susceptibility. In addition, rs5186 of *AGTR1,* rs3744700 of *CXCL16* and rs7291467 of *LGALS2* were all related to the risk for CHD.

Association studies investigating interactions of multiple genes and CHD have indicated that CHD occurrence is related to combined effects of genetic polymorphisms, suggesting there exists gene-gene interactions. In our study, we demonstrated that genes of *PPARG, AGTR1, CXCL16* and *LGALS2* may interact each other and work together in the occurrence of CHD. Further research on gene-gene interaction associated with CHD susceptibility is needed to provide more precise evidence for the issue.

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Disclosure of conflict of interest

None.

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