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Original Article

Impact of *PPAP2B* and *TCF21* gene polymorphisms on risk of coronary heart disease in Chinese Han population

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Abstract: Coronary heart disease (CHD) is the leading cause of death and disability worldwide and heritable factors account for 40 to 60% of the individual differences in CHD susceptibility. Previous study has shown that *PPAP2B* and *TCF21* involved in coronary vascular development. But little is known about single nucleotide polymorphisms (SNPs) of *PPAP2B* and *TCF21* associated with CHD. We conducted a case-control study among 597 cases and 708 healthy controls from northwest China. Eleven SNPs were selected and genotyped using Sequenom Mass-ARRAY technology. Odds ratios and 95% confidence intervals (CIs) were calculated by unconditional logistic regression adjusting for age and gender. We identified that the minor allele of rs1759752 (OR = 1.306; 95% CI = 1.070-1.595; *P* = 0.009) was significantly associated with increasing CHD risk in allelic model analysis. In the genetic model analysis, we found the "G/C-C/C" genotype of rs1759752 was associated with increased CHD risk in the dominant model (*P* = 0.028); the "G/G" genotype of rs2327433 was significantly associated with increased CHD risk based on the codominant model (*P* = 0.03) and recessive model (*p* = 0.009). In contrast, the "G/A" genotype of rs2327433 was associated with decreased CHD risk under the codominant model (*P* = 0.03). Our data shed new light on the association between *PPAP2B* and *TCF21* SNPs and CHD susceptibility in the Han Chinese population.

Keywords: Coronary heart disease (CHD), single nucleotide polymorphism (SNP), *PPAP2B, TCF21*, case-control study

Introduction

Coronary heart disease (CHD), including myocardial infarction, angina pectoris, and aterios sclerosis of the coronary arteries, is the leading causes of death and disability worldwide [1]. In China, the morbidity rate of CHD was 1.59% in cities, 0.48% in countryside; and the north China is higher than south. Though CHD's etiology seems to be multi-factorial and complicated, evidence is increasing that genetic factors have a significant role in disease etiology [2-4]. It has been proved that heritable factors account for 40 to 60% of the individual differences in CHD susceptibility [5].

Over the past years, a lot of genomic regions, polymorphisms in candidate genes have been identified associated with the risk of CHD [6-8]. The CHD gene database includes information on more than 300 candidate genes [9], but most of the variants and genes have not been established consistently. This provided us opportunity to identify genetic polymorphisms we interested associated with CHD. *PPAP2B*, encoding the Lipid phosphate phosphatase 3 (LPP3), which is not only required for vascular development but may also mediate human atherosclerotic disease [10]. Previous study have identified rs17114046 in the final intron of *PPAP2B* that associate with increased risk for CHD [11], but few study is concerning other single nucleotide polymorphisms (SNPs) in *PPAP2B*. Additionally, *TCF21* is a member of the basic helix-loop-helix transcription factor family and regulates cell fate decisions and differentiation during development of the coronary vasculature [12, 13]. Rs12190287 in *TCF21* was first identified associated with CHD in European ancestry [6], but was not replicated in another

Table 1. Characteristics of cases and controls in this study

Variables	Case (N = 597)	Control (N = 688)	Total	*p*-value
Sex, No. (%)				0.016[a]
Male	376 (63)	388 (56.4)	746 (59.5)	
Female	221 (37)	300 (43.6)	521 (40.5)	
	597	688		
Mean age ± SD	61.44 ± 11.15	48.56 ± 9.55		< 0.001[b]

[a]*P* values was calculated from Pearson's chi-square tests. [b]*P* value was calculated by Welch's t tests.

study in a Han Chinese population [8], and few information is found about other SNPs on *TCF21* associated with CHD risk.

To assess the association between SNPs in *PPAP2B* and *TCF21* and the risk of CHD in the Chinese Han population, we genotyped eleven SNPs of *PPAP2B* and *TCF21* in a case-control study with 597 cases and 708 controls from northwest China. Our data shed new light on the association between *PPAP2B* and *TCF21* SNPs and CHD susceptibility in the Han Chinese population.

Materials and methods

Study participants

All participants in our study were Han Chinese. A total of 597 patients and 708 controls were consecutively recruited between January 2011 and December 2014 at the Dongguan Branch Yan'an University Affiliated Hospital in Yan'an, China. There were no gender, age, or disease-stage restrictions for case recruitment. All cases were previously healthy. Coronary heart disease (CHD) was confirmed by standard coronary angiography. Subjects with myocardial infarction, stable angina and unstable angina were classified as CHD subjects. Subjects with congenital heart disease, familial hypercholesterolemia, end-stage renal disease and known vasculitides were excluded. Controls were selected unrelated and randomly. Among control individuals, none had any chronic or severe endocrinological, metabolic, or nutritional diseases. Basic characteristics of the participants, e.g., gender and age are listed in **Table 1**.

Demographic and clinical data

We used a standard epidemiological questionnaire and in-person interview to collect personal data, including information on residential regions, age, gender, education status, and family history of cancer. Detailed clinical information on cases was collected from treating physicians or medical chart re-views. All of the participants signed an informed consent agreement. The Human Research Committee for Approval of Research Involving Human Subjects, Dongguan Branch Yan'an University Affiliated Hospital, approved the use of human tissue in this study.

SNP selection and genotyping

Among the 11 SNPs we selected, rs12190287 was chosen from previously published polymorphisms associated with CHD [6], others were randomly chosen from the published genes (*PPAP2B* and *TCF21*) associated with CHD [8, 11]. Minor allele frequencies of all SNPs were > 5%, in the HapMap of the Chinese Han Beijing (CHB) population. DNA was extracted from whole-blood samples by GoldMag-Mini Whole Blood Genomic DNA Purification Kit (GoldMag Co. Ltd. Xi'an City, China). Quantification of the extracted DNA was performed using NanoDrop 2000. The multiplexed SNP MassEXTENDED assay was designed using Sequenom MassARRAY Assay Design 3.0 Software [14]. Genotyping was done with the Sequenom MassARRAY RS1000 system using the standard protocol recommended by the manufacturer. Data management and analysis was done using Sequenom Typer 4.0 Software [14, 15].

Statistical analysis

We used Microsoft Excel and the SPSS 18.0 statistical package (SPSS, Chicago, IL, USA) to perform statistical analyses. All *p* values presented in this study are two sided, and $P = 0.05$ was considered the cutoff for statistical significance. The differences in the characteristics of the case and control study populations were compared using the chi-squared test (for categorical variables) and Welch's t tests (for continuous variables). In all analyses, the lower frequency allele was coded as the 'risk' allele. Control genotype frequencies for each SNP were tested for departure from Hardy-Weinberg equilibrium (HWE) using Fisher's exact test. The χ^2 test was used to compare genotype frequencies in cases and controls [16]. The effects of the polymorphisms on the risk of CHD were

Table 2. Allele frequencies in cases and controls and odds ratio estimates for coronary heart disease

SNP ID	Gene (s)	Band	Alleles A[a]/B	MAF		H-W *p*-value	ORs	95% CI	*P* value
				Case	Control				
rs11206831	PPAP2B	1p32.2	T/C	0.050	0.050	1.000	1.002	0.703-1.429	0.990
rs1759752	PPAP2B	1p32.2	C/G	0.207	0.166	1.000	1.306	1.070-1.595	0.009*
rs1930760	PPAP2B	1p32.2	T/C	0.483	0.483	0.819	1.003	0.859-1.171	0.972
rs12566304	PPAP2B	1p32.2	A/C	0.151	0.148	1.000	1.018	0.819-1.265	0.872
rs914830	PPAP2B	1p32.2	T/C	0.341	0.339	0.233	1.010	0.857-1.190	0.904
rs1572425	TCF21	6q23.2	C/T	0.087	0.086	0.809	1.001	0.760-1.319	0.995
rs2327431	TCF21	6q23.2	T/C	0.087	0.086	0.466	1.001	0.767-1.330	0.945
rs6569916	TCF21	6q23.2	A/G	0.078	0.077	0.789	1.012	0.757-1.352	0.936
rs2327433	TCF21	6q23.2	G/A	0.159	0.148	0.070	1.087	0.877-1.348	0.445
rs12190287	TCF21	6q23.2	G/C	0.372	0.398	0.811	0.895	0.763-1.050	0.173
rs7766238	TCF21	6q23.2	A/G	0.085	0.085	0.214	1.000	0.755-1.326	0.998

[a]Minor allele; *p value ≤ 0.05 indicates statistical significance; MAF, minor allelic frequency; HWE, Hardy-Weinberg Equilibrium; ORs, odds ratios; CI: confidence interval.

Table 3. Logistic regression analysis of the association between the SNPs and coronary heart disease (adjusted by sex and age)

SNP	Model	Genotype	Case	Control	OR (95% CI)	*P*-value	AIC	BIC
rs1759752	Codominant	G/G	371 (62.1%)	474 (69.5%)	1	0.081	1332.8	1358.5
		G/C	205 (34.3%)	189 (27.7%)	1.34 (1.01-1.79)			
		C/C	21 (3.5%)	19 (2.8%)	1.61 (0.76-3.40)			
	Dominant	G/G	371 (62.1%)	474 (69.5%)	1	0.028*	1331	1351.6
		G/C-C/C	226 (37.9%)	208 (30.5%)	1.36 (1.03-1.80)			
	Recessive	G/G-G/C	576 (96.5%)	663 (97.2%)	1	0.310	1334.8	1355.4
		C/C	21 (3.5%)	19 (2.8%)	1.47 (0.70-3.08)			
	Overdominant	G/G-C/C	392 (65.7%)	493 (72.3%)	1	0.063	1332.3	1353
		G/C	205 (34.3%)	189 (27.7%)	1.31 (0.99-1.74)			
	Log-additive	---	---	---	1.32 (1.03-1.67)	0.026*	1330.8	1351.4
rs2327433	Codominant	A/A	429 (71.9%)	493 (71.7%)	1	0.030*	1335	1360.8
		G/A	146 (24.5%)	186 (27%)	0.93 (0.69-1.26)			
		G/G	22 (3.7%)	9 (1.3%)	3.19 (1.26-8.04)			
	Dominant	A/A	429 (71.9%)	493 (71.7%)	1	0.840	1340	1360.6
		G/A-G/G	168 (28.1%)	195 (28.3%)	1.03 (0.77-1.38)			
	Recessive	A/A-G/A	575 (96.3%)	679 (98.7%)	1	0.009*	1333.2	1353.8
		G/G	22 (3.7%)	9 (1.3%)	3.25 (1.29-8.17)			
	Overdominant	A/A-G/G	451 (75.5%)	502 (73%)	1	0.480	1339.5	1360.2
		G/A	146 (24.5%)	186 (27%)	0.90 (0.66-1.21)			
	Log-additive	---	---	---	1.13 (0.88-1.46)	0.340	1339.1	1359.7

AIC: Akaike's Information criterion; BIC: Bayesian Information criterion; *p value ≤ 0.05 indicates statistical significance.

expressed as odds ratios (ORs) with 95% confidence intervals (95% CIs), computed using unconditional logistic regression analysis with adjustments for age and sex [17].

Associations between SNPs and risks of CHD were tested in genetic models by analysis with SNP Stats software, obtained from http://bioinfo.iconcologia.net. Values of OR and 95% CI were calculated as above. Akaike's Information Criterion (AIC) and Bayesian Information Criterion (BIC) were applied to choose the best-fit model for each SNP. Pairwise linkage disequilibrium and haplotype construction were

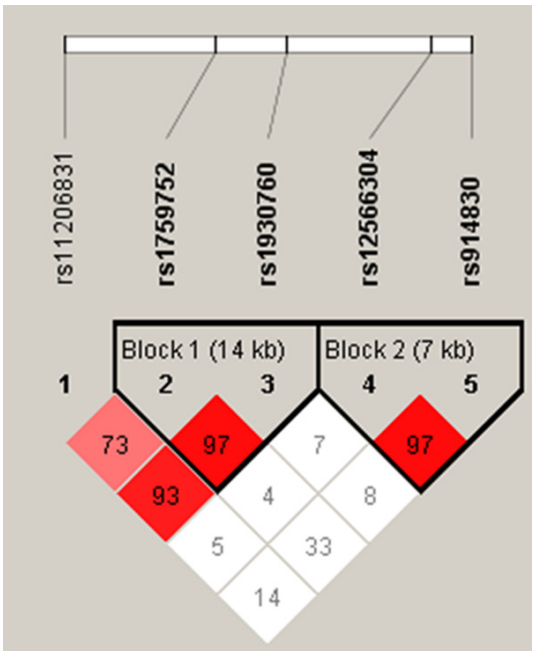

Figure 1. Linkage disequilibrium (LD) of polymorphic sites in the PPAP2B gene. A standard color scheme is used to display LD with bright red for very strong LD (LOD = 2, D' = 1), white for no LD (LOD < 2, D' < 1), pink red (LOD = 2, D' < 1), and blue (LOD < 2, D' = 1) for intermediate LD.

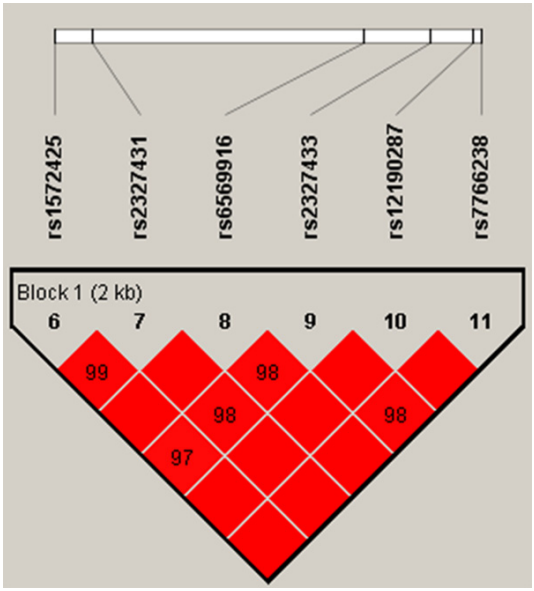

Figure 2. Linkage disequilibrium (LD) of polymorphic sites in the TCF21 gene. A standard color scheme is used to display LD with bright red for very strong LD (LOD = 2, D' = 1), white for no LD (LOD < 2, D' < 1), pink red (LOD = 2, D' < 1), and blue (LOD < 2, D' = 1) for intermediate LD.

performed using the SHEsis software (http://analysis.bio-x.cn/myAnalysis.php) [18].

Results

A total of 597 cases and 688 controls were enrolled in our study. **Table 1** showed the characteristics of cases and controls. There was no significant difference in gender distribution between the case and control groups; but it does exist in age distribution ($P < 0.001$).

Table 2 summarized the minor allelic frequency (MAF) of tested SNPs among the individuals in the case and control groups. All the SNPs were in Hardy-Weinberg equilibrium (HWE) in controls ($P > 0.05$). The allelic frequency of SNPs in the controls group was similar to those of the HapMap Asian population. Through the χ^2 test, we found that rs1759752 (OR = 1.306; 95% CI = 1.070-1.595; $P = 0.009$) was significantly associated with increasing CHD risk.

Furthermore, we assumed that the minor allele of each SNP as a risk factor compared with the wild-type allele. Five genetic models (codominant, dominant, recessive, overdominant, and additive) were applied to analyze the associations between the SNPs and CHD risk using a logistic regression test. We found that the "G/C-C/C" genotype of rs1759752 was associated with increased CHD risk, based on the dominant model (OR = 1.36; 95% CI = 1.03-1.80; $P = 0.028$). Additionally, the "G/G" genotype of rs2327433 was significantly associated with increased CHD risk, based on the codominant model (OR = 3.19; 95% CI = 1.26-8.04; $P = 0.03$) and recessive model (OR = 3.25; 95% CI = 1.29-8.17; $P = 0.009$). In contrast, the "G/A" genotype of rs2327433 was associated with decreased CHD risk, based on the codominant model (OR = 0.93; 95% CI = 0.69-1.26; $P = 0.03$) (**Table 3**).

Three blocks were detected in studied *PPAP2B* and *TCF21* SNPs by haplotype analyses (**Figures 1**, **2**). The results of the association between the *PPAP2B* and *TCF21* haplotype and the risk of CHD were listed in **Table 4**. Haplotype "GC" and "CC" of *PPAP2B* in Block 1 were found to be associated with decrease and increase risk of CHD, respectively ($p_1 = 0.033$; $p_2 = 0.01$). However, the *P*-values were not significant after adjusting by age and sex.

Discussion

In the present case-control study, we investigated the association between eleven SNPs of

Table 4. PPAP2B and TCF21 haplotype frequencies and the association with the risk of coronary heart disease in case and control patients

Gene	Block	Haplotype	Freq (case)	Freq (control)	χ^2	Pearson's *P*	OR	[95% CI]	*P*-value[a]
PPAP2B	1	GT	0.479	0.480	0.003	0.960	1 (referent)	/	/
		GC	0.314	0.354	4.552	0.033*	0.88	0.71-1.09	0.23
		CC	0.203	0.164	6.731	0.010*	1.24	0.96-1.60	0.11
	2	CC	0.508	0.514	0.104	0.747	1 (referent)	/	/
		CT	0.341	0.338	0.035	0.851	1.09	0.88-1.34	0.43
		AC	0.151	0.148	0.041	0.840	0.98	0.75-1.28	0.87
TCF21	1	TCGACG	0.469	0.453	0.690	0.406	1 (referent)	/	/
		TCGAGG	0.372	0.400	2.120	0.146	0.88	0.71-1.07	0.20
		CTAGCA	0.078	0.076	0.035	0.851	1.02	0.71-1.47	0.92
		TCGGCG	0.071	0.063	0.726	0.394	1.12	0.76-1.64	0.57

*p value ≤ 0.05 indicates statistical significance; [a]adjusted by sex and age; OR: odd ratio; CI: confidence interval.

PPAP2B and *TCF21* and the risk of CHD in the Chinese Han population. Among the eleven SNPs, the "G/C-C/C" genotype of rs1759752 in *PPAP2B* and the "G/G" genotype of rs2327433 in *TCF21* were found to be associated with increased CHD risk. In contrast, the "G/A" genotype of rs2327433 was associated with decreased CHD risk. Additionally, we detect three blocks in studied SNPs by haplotype analyses, but none of the haplotype was significantly associated with risk of CHD.

There are three lipid phosphate phosphatases (LPPs) in mammals. *PPAP2A*, *PPAP2C*, and *PPAP2B* genes encoding LPP1, LPP2, and LPP3, respectively. Previous study have shown that gene-trap inactivation of *PPAP2C* [19] and loss of *PPAP2A* [20] in mice does not result in phenotypic alteration. However, global knockout of *PPAP2B* in mice results in embryonic lethality, mainly because of defects in extraembryonic vascular development [10]. These studies make the association of *PPAP2B* with CHD is particularly compelling. However, the most robust genetic risk variant for CHD was rs17114046 identified in the final intron of *PPAP2B* by genome-wide association study [11]. In our study, we identified rs1759752 in *PPAP2B* is associated with CHD with a 1.306-fold of CHD risk. To the best of our knowledge, this is the first time for reporting this SNP associated with CHD. Considering the insufficient methods in the laboratory as well as small scale studies, this result should be confirmed in further studies.

Previous study have shown that inactivation of *TCF21* in mouse results in increased expression of coronary vascular smooth muscle cell as well as premature differentiation [21], and a dramatic failure of cardiac fibroblast development [22]. Rs12190287 at 6q23.2, located within the 3' untranslated region (3'UTR) of *TCF21*, was first reported associated with CHD at European populations [6], but later was not successfully replicated in the Chinese Han population [8]. In our study, we did not detect any associations between rs12190287 and CHD in the Chinese Han population. These data suggesting that *TCF21* may have different disease mechanisms in CHD development in diverse populations, or that the associations are not significant since the sample size is relatively small. We also identified the rs2327433 in *TCF21*, for the first time, associated with CHD risk. In fact, studies on this loci are rare, we will do further studies in a larger sample size to confirm it.

Despite the current study possessing enough power, some limitations should be considered. As we all know, CHD are very heterogeneous disease and have many traditional risk factors, including tobacco use, physical inactivity, poor nutrition, obesity, hypertension, high blood cholesterol, and diabetes. Because the sample size of our study was relatively small and the data of detail information was absent, we could not explore how genetic polymorphisms and these factors interact in CHD. So the gene-gene and gene-environment interactions of *PPAP2B* and *TCF21* need to be evaluated in future studies.

In conclusion, our study has described the association between rs1759752 (*PPAP2B*) and

rs2327433 (*TCF21*) and CHD risk in Chinese Han population. Although we did not find any interesting results, this is the first report on the association between rs1759752 and rs2327433 and the risk of CHD.

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Disclosure of conflict of interest

None.

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