

# Association Study of Single-Nucleotide Polymorphisms on Chromosome 1p13, 1p32, 9p21 and 19p13 with Cardiovascular Diseases in Chinese Han Population: A Case-Control Study

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## Abstract

**Objectives:** Previous research discovered single nucleotide polymorphism (rs2383206 and rs2383207) on chromosome 9p21 that is associated with coronary heart disease in a Chinese population. However, few data are available on the association of other single nucleotide polymorphism with cardiovascular disease in a Chinese population. This study aimed to determine whether the single nucleotide polymorphisms on chromosome 1p13, 1p32, 9p21 and 19p13 were associated with coronary artery disease in a Chinese population.

**Methods:** We conducted a case-control study. Cases were coronary artery disease (n=670) between 2010 and 2015. Controls (n=1340) were randomly selected and frequency matched to cases on age and gender. All of the participants were selected to study 18 single nucleotides using allele-specific real-time polymerase chain reaction method.

**Results:** Four single nucleotides in 9p21, two single nucleotides in 1p13 and one single nucleotide in 1p32 were associated with cardiovascular disease risk in Chinese population (Global P value for multiple logistic regression, <0.0001, respectively). rs10757274 showing the strongest association with cardiovascular disease. GG carriers of four SNPs (rs10757274, rs2383206, rs10757278 and rs1333049) in 9p21 had higher risk (Odds ratio=1.40, 95% Confidence interval: 1.10-1.79; Odds ratio=1.33, 95% Confidence interval: 1.04-1.69; Odds ratio=1.35, 95% Confidence interval: 1.07-1.72; Odds ratio=1.34, 95% Confidence interval: 1.06-1.71).

**Conclusion:** rs10757274, rs2383206, rs10757278 in 9p21, rs562556 in 1p32, and rs646776 in 1p13 may serve as a novel genetic marker for the risk of significant cardiovascular disease in Chinese Han population.

**Keywords:** Cardiovascular disease; Coronary artery disease; Single-nucleotide polymorphisms; Haplotype; Chinese population

## Introduction

Cardiovascular disease (CVD) is the leading cause of death and disability-adjusted life-years worldwide, with increasing incidence and prevalence in low and middle income countries [1]. Non-modifiable risk factors include increasing age, male sex, and heredity. Modifiable risk factors include smoking, hypertension, dyslipidemia, obesity, physical inactivity, and diabetes [2-4].

In 2007, genome-wide association studies on CVD identified a series of associated single-nucleotide polymorphisms (SNPs) in an intergenic region of chromosome 9p21, near the CDKN2A and CDKN2B genes [5-7]. In Chinese Han population few studies have simultaneously explored the relationship of these and other genes on risk of coronary artery disease (CAD). Despite recent progress in identifying some novel genetic contributors to CVD, it is currently unknown how these recently discovered loci interact with the environment and what role such interactions play in the development of disease [8,9]. Investigation of gene-environment interactions are necessary to further our understanding of the underlying biology and

pathophysiology of the disease, and could potentially be useful in improving cardiovascular risk stratification and thereby reducing clinical events [9-11]. Our study has been carried out in Beijing of China, showed that nine potentially modifiable risk factors account for most of the risk of CAD. We sought to investigate this further by first, exploring the risk genes in Chinese Han population and assessing whether the relationships between risk genes and CAD, and second, identifying some genetic and other risk factors interactions and comparing the variations in risk genes and CAD relationships between different stratification.

We examined the association of common genetic variation in 1p32 near PCSK9, 1p13 near CELSR2-PSRC1-SORT1, 9p21 near CDKN2A, CDKN2B, and 19p13 near LDLR with risk of incident acute myocardial infarction in a population-based case-control study.

## Methods

### Study populations

The setting for this study has been carried out in Beijing of China. The affiliated hospital of Capital Medical University coordinated the study. A case was defined as patients with angiographically confirmed

narrowing of the coronary vessels by more than 50%, fatal or nonfatal myocardial infarction or unstable angina pectoris, as well as evidence of CAD by coronary angiography. Patients with congenital heart disease, cardiomyopathy, valvular heart disease, and renal or hepatic disease were excluded from the study. At least two age ( $\pm 5$  years) control (without a history of CVD) was recruited for each case. Every participant was assigned an index date. For cases, the index date was the admission date for CAD. For the controls, the index date was a random date within the year for which they were sampled. In addition, we excluded patients whose blood specimens did not yield genotype information. The study was approved by the ethics committee of Capital Medical University (No.2015SY52), and written informed consent was obtained from each subject before his/her inclusion in the study.

### Epidemiological survey

Trained personnel administered the structured questionnaires and physical examinations in a standardized manner. We use of standardized questionnaires for all study participants to obtain the information, containing demographic factors, lifestyle, smoking history, alcohol use, family income and education, psychosocial factors, physical activities, personal and family history of CVD and risk factors. Blood pressure, height, weight, waist and hip circumferences were measured. Body mass index (BMI) was defined as weight (kg) divided by height squared ( $m^2$ ). Questions were included about psychosocial conditions to identify psychological stress. General psychological stress was defined as experiencing stress at work or at home and was also assessed in the four categories.

### Genetic variation

Single nucleotide polymorphisms (SNPs) and Haplotype were identified in two regions (1p13 near CELSR2-PSRC1-SORT1 and 9p21 near CDKN2A, CDKN2B). Among variants, SAS/genetics was selected maximally informative sets of SNPs to describe genetic variation in Chinese Han population using linkage disequilibrium and of 0.90.

### Blood collection and genotyping

Non-fasting blood samples (20 ml) were drawn and centrifuged within 2 h of admission, frozen immediately after processing. Samples were shipped in nitrogen vapour tanks from every site to a blood storage site and stored at  $-70^\circ C$  in freezers or  $-170^\circ C$  in liquid nitrogen.

A total of 2010 participants (670 CAD cases and 1340 controls) were recruited for our study, and of these, all participants had DNA samples available. The SNPs was genotyped by allele-specific real-time polymerase chain reaction (PCR) using GeneAmp 5700 Sequence Detector (Applied Biosystem, Foster city, CA, USA). We obtained genotyping results on the study subjects.

### Statistical analysis

Continuous variables differences between cases and controls were calculated by t test. Categorical variables differences were calculated by Pearson chi-squared analysis. Hardy-Weinberg equilibrium was also assessed by Pearson chi-squared analysis in the ALLELE procedure in SAS software package (Version 9.3; SAS Institute, Chicago, IL, USA)/Genetics.

We assessed genotypes and allele frequencies difference between CAD and control group using Pearson chi-squared, Fisher's exact tests and Trend test in the CASECONTROL procedure of SAS/Genetics. Haplotype frequencies were inferred using the Expectation-Maximization algorithm available in the HAPLOTYPE procedure in SAS/Genetics. Haplotype figure was described by Haploview software.

Odds ratios estimates and 95% confidence intervals for each variant were calculated in logistic regression models. Adjusted odds ratios were performed by multiple logistic regressions, and it controls for gender, age, obesity, smoking, alcohol, type 2 diabetes, stroke history, stress, Apolipoprotein-A1 (ApoA1), and Apolipoprotein-b (ApoB). Data were analysed using LOGISTIC procedure in SAS/STAT. Wald test and likelihood-ratio test calculated the statistical significance.  $P < 0.05$  was reject level to indicate statistically significant differences.

### Results

#### Characteristics of participants

The general and biochemical characteristics between CAD cases and controls are detailed in (Table 1). The levels of body mass index (BMI), waist-to-hit ratio (WHR), ApoB, systolic blood pressure, and diastolic blood pressure and the percentages of general stress were higher in CAD group than control group ( $P < 0.05-0.001$ ). There were no significant differences the percentages of subjects who smoked cigarettes and consumed alcohol ( $P > 0.05$  for all).

Characteristics	CVD cases, N=670	controls, N=1340	t	P-value
Male sex (%)	425 (63.5)	976 (72.8)	5.83	0.0158*
Mean Age (SD), year	64.9 (10.2)	60.0 (10.5)	5.53	0.0001*
BMI, mean (SD), kg/cm <sup>2</sup>	25.2 (2.8)	24.0 (2.9)	5.49	0.0001*
WHR, mean (SD), cm/cm	0.90 (0.09)	0.86 (0.08)	4.7	0.0001*
Systolic blood pressure (mmHg)	142.3 (17.9)	123.1 (12.7)	13.34	0.0001*
Diastolic blood pressure (mmHg)	86.1 (10.7)	77.5 (7.5)	10.06	0.0001*
Type 2 diabetes (%)	73 (10.9)	16 (1.2)	32.49	0.0001*
Stroke (%)	80 (11.9)	24 (1.8)	29.28	0.0001*

Smoking, n (%)	248 (37.0)	531 (39.6)	0.42	0.5146
Alcohol, n (%)	74 (10.9)	197 (14.5)	1.61	0.205
General stress, n (%)			8.15	0.0170*
Permanent	245 (36.5)	352 (26.3)		
Several periods	279 (41.7)	697 (52.0)		
Never experienced	146 (21.8)	291 (21.7)		
ApoA1 (g/L)	1.33 (0.30)	1.40 (0.32)	-2.48	0.0133*
ApoB (g/L)	0.87 (0.21)	0.82 (0.22)	3.05	0.0023*
ApoA1/ApoB	1.60 (0.45)	1.80 (0.59)	-4.66	0.0001*

N-number; p-value, significance of difference between group means or frequencies determined by t-test or chi-square test; BMI: body mass index; WHR: waist-to-hip ratio; ApoA1: Apolipoprotein-A1; ApoB: Apolipoprotein-B \*Considered statistically significant

**Table 1:** Characteristics of participants.

### SNPs genotype and their frequencies

We genotyped three informative SNPs in 1p13 near CELSR2-PSRC1-SORT1, eight SNPs in 9p21 near CDKN2A, CDKN2B and other seven SNPs. These SNPs were in Hardy-Weinberg equilibrium in CAD and controls except rs646776. In the Uni-variable Logistic regression, eight SNPs in 1p13 and 9p21 were associated with CAD. Next, we adjusted CAD risk factors BMI, smoking, drink, Hypertension, Diabetes, Stroke, CVD of family, stress. Upon adjustment, the effect of the rare alleles of the analysed SNPs remained highly significant and was similar to that seen in the unadjusted analysis. Five SNPs in 9p21 and two SNPs in 1p13 showed the

association under the multiplicative model. rs10757274 showing the strongest association. For the G allele frequency at rs10757274 in CAD, was significantly higher than control group (50.6% vs. 46.1%,  $P=0.002$ , respectively). We found that each additional copy of the G allele at rs10757274 increased the risk of CAD (OR: 1.182, 95%CI: 1.048-1.333), when compared to the A allele homozygotes. GG carriers of four SNPs (rs10757274, rs2383206, rs10757278 and rs1333049) in 9p21 had higher risk (OR=1.40, 95%CI: 1.10-1.79; OR=1.33, 95%CI: 1.04-1.69; OR=1.35, 95%CI: 1.07-1.72; OR=1.34, 95%CI: 1.06-1.71) (Table 2).

SNP	Control		CVD		Chr	Pos	Nearby gene	P value <sup>a</sup>	Crude OR(CI) <sup>b</sup>	Adjusted OR(CI) <sup>c</sup>
	count	(%)	count	(%)						
rs11206510					1p32	5.5E+07	PCSK9	0.576		
GG	5	0.4	1	0.2					1	1
AG	155	11.6	72	10.7					1.78 (0.32-9.89)	1.26 (0.22-7.30)
AA	1180	88.1	597	89.2					1.96 (0.36-10.73)	1.35 (0.24-7.68)
rs11591147					1p32	5.6E+07	PCSK9	0.348		
CC	1241	92.6	624	93.1					1	1
AC	99	7.4	45	6.7					0.90 (0.65-1.23)	0.83 (0.59-1.16)
AA	0	0	1	0.2						
rs562556					1p32	5.6E+07	PCSK9	0.651		
GG	3	0.2	5	0.8					1	1
AG	34	2.5	11	1.7					0.15 (0.03-0.75)	0.12 (0.02-0.63)
AA	1303	97.3	653	97.5					0.22 (0.05-1.00)	0.20 (0.04-0.94)
rs505151					1p32	5.6E+07	PCSK9	0.223		
AA	1189	88.7	582	87					1	1

AG	146	10.9	85	12.7					1.18 (0.92-1.52)	1.20 (0.92-1.56)
GG	5	0.4	3	0.4					1.05 (0.26-4.21)	0.90 (0.21-3.90)
rs611917					1p13	1.1E+08	CELSR2- PSRC1- SORT1	0.067		
GG	4	0.3	2	0.3					1	1
AG	189	14.1	69	10.3					0.71 (0.14-3.56)	0.74 (0.13-4.10)
AA	1147	85.6	599	89.4					1.01 (0.20-5.03)	1.09 (0.20-5.93)
rs646776					1p13	1.1E+08	CELSR2- PSRC1- SORT1	0.016 <sup>*</sup>		
AA	1030	76.9	547	81.7					1	1
AG	257	19.2	103	15.4					0.76 (0.61-0.94)	0.79 (0.63-0.99)
GG	52	3.9	20	2.9					0.70 (0.44-1.10)	0.69 (0.43-1.12)
rs602633					1p13	1.1E+08	CELSR2- PSRC1- SORT1	5×10 <sup>-3*</sup>		
AA	4	0.3	2	0.3					1	1
AC	192	14.3	64	9.6					0.65 (0.13-3.28)	1.08 (0.18-6.41)
CC	1144	85.4	604	90.2					1.02 (0.21-5.08)	1.74 (0.30-10.20)
rs7044859					9p21.3	2.2E+07	CDKN2A, CDKN2B	0.059		
TT	532	39.7	302	45.1					1	1
AT	647	48.3	294	43.9					0.80 (0.67-0.95)	0.81 (0.68-0.97)
AA	161	12	74	11					0.81 (0.62-1.06)	0.85 (0.64-1.12)
rs564398					9p21.3	2.2E+07	CDKN2A, CDKN2B	0.021 <sup>*</sup>		
AA	1017	75.9	534	79.8					1	1
AG	308	23	132	19.7					0.82 (0.67-0.99)	0.81 (0.66-1.00)
GG	15	1.1	4	0.5					0.38 (0.13-1.06)	0.44 (0.15-1.31)
rs1333040					9p21.3	2.2E+07	CDKN2A, CDKN2B	0.053		
GG	124	9.2	43	6.4					1	1
AG	549	41	278	41.5					1.47 (1.06-2.03)	1.52 (1.08-2.13)
AA	667	49.8	349	52.1					1.52 (1.11-2.09)	1.56 (1.11-2.18)
rs10757274					9p21.3	2.2E+07	CDKN2A, CDKN2B	6×10 <sup>-3*</sup>		
AA	389	29	171	25.5					1	1
AG	666	49.7	320	47.8					1.10 (0.90-1.34)	1.11 (0.90-1.36)
GG	285	21.3	179	26.7					1.43 (1.14-1.80)	1.40 (1.10-1.79)
rs2383206					9p21.3	2.2E+07	CDKN2A, CDKN2B	0.022 <sup>*</sup>		
AA	373	27.8	167	24.9					1	1
AG	666	49.7	322	48.1					1.09 (0.89-1.32)	1.09 (0.89-1.34)
GG	301	22.5	181	27					1.34 (1.07-1.68)	1.33 (1.04-1.69)
rs10757278					9p21.3	2.2E+07	CDKN2A, CDKN2B	0.013 <sup>*</sup>		
AA	363	27.1	159	23.8					1	1

AG	658	49.1	322	48					1.11 (0.91-1.36)	1.13 (0.91-1.39)
GG	319	23.8	189	28.2					1.34 (1.07-1.69)	1.35 (1.07-1.72)
rs1333049					9p21.3	2.2E+07	CDKN2A, CDKN2B	0.016*		
CC	358	26.7	156	23.3					1	1
CG	661	49.3	327	48.8					1.14 (0.93-1.39)	1.14 (0.92-1.41)
GG	321	24	187	27.9					1.33 (1.06-1.68)	1.34 (1.06-1.71)
rs10811661					9p21.3	2.2E+07	CDKN2A, CDKN2B	0.419		
GG	306	22.8	143	21.3					1	1
AG	669	49.9	329	49.1					1.05 (0.86-1.30)	1.01 (0.82-1.26)
AA	366	27.3	198	29.6					1.17 (0.93-1.47)	1.10 (0.86-1.40)
rs6511720					19p13	1.1E+07	LDLR	0.143		
CC	1317	98.3	662	98.8					1	1
AC	23	1.7	8	1.2					0.72 (0.36-1.43)	0.57 (0.27-1.21)
AA	0	0	0	0						
rs6511721					19p13	1.1E+07	LDLR	0.845		
GG	97	7.2	47	7					1	1
AG	505	37.7	251	37.5					1.02 (0.73-1.43)	1.06 (0.75-1.50)
AA	738	55.1	372	55.5					1.04 (0.75-1.44)	1.06 (0.75-1.48)
rs1433099					19p13	1.1E+07	LDLR	0.263		
GG	707	52.8	367	54.8					1	1
AG	527	39.3	249	37.2					0.91 (0.77-1.08)	0.90 (0.75-1.08)
AA	106		54	8						

Chr: Chromosome; Pos: position; SNP: single nucleotide polymorphism; OR: odds ratio; CI: confidence interval;  
<sup>a</sup> P-value, Multivariable model controls for BMI, smoking, drink, Hypertension, Diabetes, Stroke, CVD of family, stress;  
<sup>b</sup> Crude OR of being influenced by a SNP for an expression trait associated with a given risk factor;  
<sup>c</sup> Adjusted for BMI, smoking, drink, Hypertension, Diabetes, Stroke, CVD of family, stress;  
\* considered statistically significant.

**Table 2:** 18 SNPs genotype and their frequencies.

### Stratification analysis

We conducted stratified analysis for the three SNPs (Table 3). GG carriers of two SNPs (rs2383206 and rs10757278) had higher risk in hypertension patients (OR=1.62, 95%CI: 1.05 to 2.49, OR=1.61, 95%CI: 1.05 to 2.48, respectively). GG carriers of two SNPs (rs10757274 and rs10757278) had higher risk in BMI $\geq$ 25 kg/m<sup>2</sup>

subjects (OR=1.48, 95%CI: 1.03 to 2.13 and OR=1.48, 95%CI: 1.03 to 2.12, respectively). Furthermore, when multiplicative interaction was tested for each possible pair of these three SNPs, but we did not found significant interactions between SNP and BMI, smoking, hypertension, and diabetes.

	rs10757274 OR (95% CI)				rs2383206 OR (95% CI)				rs10757278 OR (95% CI)			
	AA	AG	GG	P	AA	AG	GG	P	AA	AG	GG	P
BMI												
25	1	1 (0.76-1.31)	1.34 (0.76-1.31)		1	1.03 (0.78-1.36)	1.26 (0.92-1.74)		1	1.03 (0.78-1.31)	1.25 (0.91-1.72)	

25	1	1.28(0.94-1.75)	1.48(1.03-2.13)	0.59	1	1.19(0.87-1.62)	1.4(0.97-2.01)	0.59	1	1.28(0.93-1.75)	1.48(1.03-2.12)	0.3 3
Smoking												
no	1	1.08(0.83-1.40)	1.52(1.21-2.07)		1	1.09(0.84-1.41)	1.4(1.03-1.90)		1	1.13(0.87-1.48)	1.51(1.12-2.05)	
yes	1	1.17(0.83-1.64)	1.25(0.85-1.83)	0.39	1	1.1(0.78-1.55)	1.2(0.82-1.78)	0.54	1	1.12(0.79-1.58)	1.11(0.76-1.63)	0.2 2
Drink												
No	1	1.02(0.78-1.34)	1.5(1.10-2.05)		1	1.04(0.80-1.36)	1.36(1.00-1.86)		1	1.12(0.85-0.47)	1.43(1.05-1.94)	
Yes	1	1.24(0.90-1.70)	1.26(0.86-1.83)	0.6	1	1.17(0.85-1.62)	1.25(0.86-1.82)	0.82	1	1.15(0.83-1.59)	1.21(0.84-1.76)	0.6 3
Hypertension												
No	1	1.06(0.83-1.37)	1.3(0.97-1.74)		1	1(0.78-1.29)	1.17(0.87-1.56)		1	1.02(0.79-1.31)	1.19(0.89-1.58)	
Yes	1	1.16(0.81-1.66)	1.53(0.99-2.36)	0.77	1	1.26(0.88-1.81)	1.62(1.05-2.49)	0.35	1	1.34(0.94-1.93)	1.61(1.05-2.48)	0.2 6
Diabetes												
No	1	1.11(0.90-1.38)	1.38(1.07-1.77)		1	1.11(0.89-1.37)	1.29(1.01-1.66)		1	1.17(0.94-1.45)	1.33(1.04-1.70)	
Yes	1	0.85(0.37-1.93)	1.41(0.54-3.72)	0.86	1	0.75(0.33-1.72)	1.48(0.56-3.91)	0.72	1	0.63(0.27-1.46)	1.32(0.50-3.52)	0.8 3

CVD: cardiovascular disease; SNP: single nucleotide polymorphism; OR: odds ratio; CI: confidence interval; BMI: body mass index.

**Table 3:** Stratification analysis for association between three SNPs in 9p21 genotypes and risk of CVD.

### Haplotype frequency

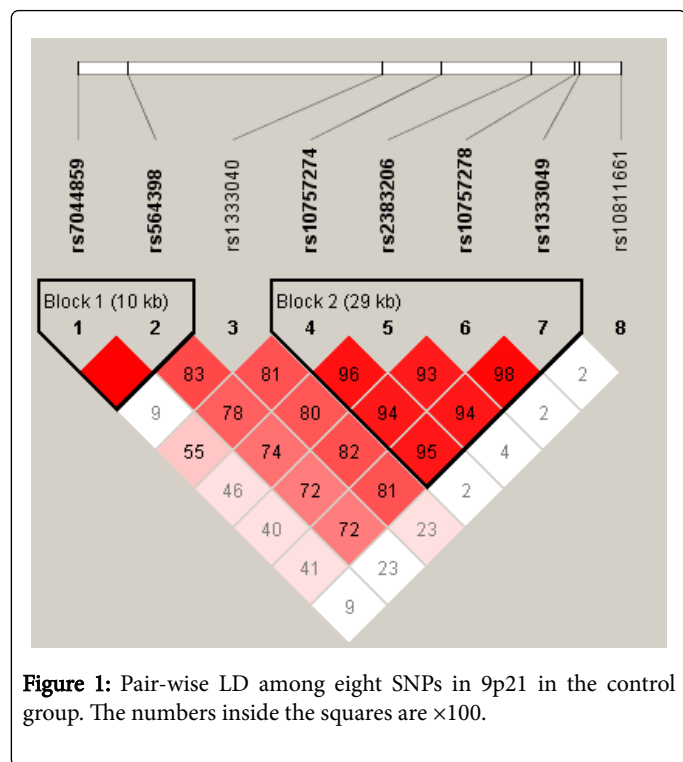


Figure 1 shows the results of haplotype analysis for the SNPs examined. And then we calculated possible haplotype frequency of 8 loci in 9p21, using haploview and SAS software. Using the genotypes of 1340 controls, we defined the haploblock structure of SNPs within the region of 9p21 in the Chinese population. By defining a solid spine of LD as  $>0.90$ , we identified one haploblock in the 9p21 region.

Next, we focused on the haplotypes within block with association. One haplotype, AAAC, was less frequent in CAD than control subjects (49.2% vs. 44.8%) and the other haplotype, GGGG, was more frequent (48.2% vs. 44.4%) (Table 4). Our results indicated that haploblock on chromosome 9p21 have possible association with CAD in the Chinese population. rs646776 and rs599839 in 1p13 have been confirmed correlation in a large scale analysis [8,9]. We chose rs611917, rs646776 and rs602633 in our study, and found that rs602633 was higher associated with risk of CAD in China population. A could be risk allele, and heterozygosis appeared in lower risk of CAD. rs646776 and rs611917 had lower association with CAD, and rs602633 was in linkage disequilibrium with rs611917 ( $r^2=0.9$ ).

Overall, common variation of four SNPs (rs10757274, rs2383206, rs10757278 and rs1333049) in 9p21 near CDKN2A, CDKN2B and two SNPs (rs602633 and rs646776) in 1p13 near CELSR2-PSRC1-SORT1 were associated with risk of CAD. We found no association of common variation of 7 SNPs in 1p32 near PCSK9 and 19p13 near LDLR with risk of CAD in Chinese population (Table 2).

Haplotype	rs10757274	rs2383206	rs10757278	rs1333049	Haplotype frequency		
					All	CVD death	Controls
Hap1	A	A	A	C	0.47	0.448	0.492
Hap2	G	G	G	G	0.463	0.482	0.444
Hap3	A	A	G	G	0.024	0.026	0.023
Hap4	A	G	G	G	0.011	0.008	0.012
Hap4	G	A	A	C	0.01	0.012	0.007
other					0.022	0.024	0.022

**Table 4:** Haplotype frequency in 9p21.

## Discussion

Genome-wide association studies have identified several SNPs as reproducibly associated with risk of CVD. SNPs at nine loci reached genome-wide significance: 21q22 near mitochondrial ribosomal protein S6 (MRPS6)- solute carrier family 5 member 3 (SLC5A3)-potassium voltage-gated channel subfamily E regulatory subunit 2 (KCNE2), 6p24 in phosphatase and actin regulator 1 (PHACTR1), 2q33 in WD repeat domain 12 (WDR12), 9p21 in CDKN2A, CDKN2B, 1p13 near CELSR2-PSRC1-SORT1, 10q11 near C-X-C motif chemokine ligand 12 (CXCL12), 1q41 in melanoma inhibitory activity family, member 3 (MIA3), 19p13 near LDLR and 1p32 near PCSK9) [7,12-15].

The region on chromosome 9p21 showed an association with the coronary heart disease (CHD) in previous studies. The associated SNPs were rs1333049 (18 researches, OR=1.23), rs10757274 (17researches, odds ratio (OR)=1.24), rs2383207 (6 researches, OR=1.28), rs2891168 (4 researches, OR=1.29), rs10757278 (2 researches, OR=1.27) [2]. In Asian race, rs1333049 (Japan, China), rs10757274 (China), rs10757278 (China) showed an association with the Myocardial infarction (MI) [2]. Our case-control study not only replicated the findings of the 3 SNPs on chromosome 9p21 that were associated with CAD, but also provided rs564398, rs2383206 that were possible associated with CAD in Chinese population. And SNP rs10757274 showed the strongest association. HapMap data suggests that rs10757274 and rs2383206 were located within 20 kb of each other on chromosome 9p21 and were in strong linkage disequilibrium ( $r^2=0.89$ ) in North American population, and the five SNPs (rs2383206, rs2383207, rs10757274, rs10757278, and rs1333049) are in 1 block, and the D' between each of the 5 SNPs was Chinese Han origin [5,16]. In our study, we identified one haploblock in the 9p21 region (rs10757274, rs2383206, rs10757278, and rs1333049) in Chinese population, and it's similar to previous North American population study. On the basis of the stringent analysis of these SNPs and replication in our research, we are confident that 9p21 region is a strong candidate locus for CAD susceptibility in the Chinese population.

CDKN2A and CDKN2B in the region of chromosome 9p21 are coding sequences for 2 cyclin-dependent kinase inhibitors. These genes involved in the regulation of the cell cycle and would be implicated, and transform growth factor (TGF)-induced growth inhibition, in the pathogenesis of atherosclerosis [16-19]. McPherson et al. re-sequenced the most proximal to the risk locus in CDKN2A and CDKN2B gene coding regions and found no association between the CHD risk and

this locus [7]. However, some researchers recently found the relationship between the same region and increased susceptibility to type 2 diabetes (T2D) [20-22]. Many studies have identified of the underlying possible mechanism at this locus of CHD. These results imply that the region of 9p21 may be associated with many complex diseases. In our study, the interactions between smoking, alcohol, and BMI with rs0757274, rs2383206, and rs10757278 were not significant, so this interaction need to be replicated in other populations.

The strongest association with CHD after 9p21 is the CELSR2-PSRC1-SORT1 gene cluster. The risk allele at CELSR2-PSRC1-SORT1 has a frequency of 81% in European subjects and an additive odds ratio of 1.19 [23,24]. rs646776 and rs599839 in 1p13 have been confirmed correlation in a large scale analysis [12,13]. Few studies have examined the associations between SNPs on chromosome 1p13 and CVD risk in East Asian populations. We found that rs646776 and rs602633 were higher associated with risk of CAD in China population. However, SNP rs646776 genotype frequencies did not obey Hardy-Weinberg equilibrium, and we thought that there was error in our experimental test and sample survey. This is limitations of our study.

The relationship of rs646776 and other SNPs at 1p13.3 with low density lipoprotein-cholesterol (LDL-C) and ApoB in Genome-wide associations study may be associated with the reported roles of the SORT1 protein in vesicular transport [25]. A recent 176 whole blood samples study implied that there exist functional variants at this locus of SORT1 gene. And the risk allele decreased expression of SORT1 gene and increased plasma LDLC concentration and the risk of CHD [22]. The gene product of SORT1 is sortilin, a multiligand receptor that may aid in the clearance of low density lipoprotein (LDL) from plasma, mediated through direct interactions with the LDL receptor-related protein [26-29]. Another 400 liver samples study confirmed the relationship of the risk allele in genome-wide associations study with increased LDLC concentrations, and these concentrations decreased SORT1 and CELSR2 expression [30]. In our study, we only found that rs646776 was associated with risk of CAD. We should increase sample size and statistical power to validate the association in other population. Meanwhile we also exclude causal roles for CELSR2, PSRC1 genes, because these genes map neighbouring recombination hotspot from SORT1 closed to the strongest associations.

9p21 in CDKN2A, CDKN2B, and 1p13 near CELSR2-PSRC1-SORT1 also showed association with other disease. Ahluwalia et al. [31] tested the 11 biomarker-associated SNPs for association with T2D in Danish samples and found significant statistical associations for

CELSR2 SNPs [OR=1.11 (1.05-1.18),  $P=1.2\times 10^{-3}$ ], respectively. rs646776 near CELSR2 was associated with an increased fasting serum insulin like growth factor binding protein 1 (IGFBP1) level and a decreased risk of T2D [31]. Gaulton KJ et al. [32] observed four loci that each had two distinct association signals, CDKN2A-CDKN2B, diacylglycerol kinase beta (DGKB), melanocortin 4 receptor, (MC4R) and gastric inhibitory polypeptide receptor, (GIPR), with each locus represented by noncoding index variants. The index variants at the CDKN2A-CDKN2B locus represent the known T2D haplotype association signal mapping to a 12-kb intergenic recombination interval [32]. In our research, we adjusted influence of type 2 diabetes on this association using by multiple regression. We will focus on this research of association between these SNPs and other disease.

In our research, we address SNP-environment interactions which are thought to underlie many diseases and traits. Using logistic model didn't find the interaction of SNPs and life style. While most researchers are eager to look at the interaction of multiple genes and environment in causing disease, the statistical tools and algorithms that are needed are still in the early stages of development.

## Conclusion

The SNP 10757274, rs2383206, rs10757278 in 9p21, rs562556 in 1p32, and rs646776 in 1p13 may serve as a novel genetic marker for the risk of significant cardiovascular disease in Chinese Han population.

## Authors' Contributions

GJ did the whole analysis of relationship of 1p32 near PCSK9, 1p13 near CELSR2-PSRC1-SORT1, 9p21 near CDKN2A, CDKN2B, and 19p13 near LDLR gene with CVD, participated in epidemiology investigation in the hospital. LH and FY did statistical analysis. GJ, ZF and LY joined the design of study and epidemiology survey. GJ, HJ, and GX joined the modification of design and helped to draft the whole manuscript. All authors approved the final manuscript.

## Competing Interests

None.

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