Contribution of IL9, IL2RA and IL2RB genetic polymorphisms in coronary heart disease in Chinese Han population

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Abstract

Background: Coronary heart disease (CHD) is one of the leading causes of disability and death worldwide. In the pathogenesis of CHD, inflammatory cytokines take an essential part. This study was designed to detect the potential association between IL-9, IL-2RA and IL-2RB variants and CHD in Chinese Han population. Methods: This case-control study conducted 499 CHD patients and 496 healthy controls. Seven selected SNPs were genotyped to investigate the possible association between the polymorphisms and the CHD risk. The interaction of SNP-SNP in the CHD risk was analyzed by Multifactor dimensionality reduction (MDR). Results: We observed an association between IL-9 rs55692658 (OR = 1.72, p = 0.003) and the increased CHD risk. The stratification analysis by age indicated that no matter participants who were older or younger than 61 years, IL-9 rs55692658 and IL-2RB rs1573673 contributed to the CHD susceptibility significantly ($p < 0.05$, respectively). IL-9 rs55692658 showed an increasing-risk effect (OR = 2.32, p = 0.003), while IL-2RA rs12722498 was correlated with the decreased susceptibility of CHD (OR = 0.54, p = 0.033) in female. Furthermore, IL-2RA rs12569923 was related to the diabetes risk in the CHD patients (OR = 1.50, p = 0.028). MDR analysis revealed a positive interaction between the SNPs. Conclusion: The present study firstly demonstrated that IL-9 rs55692658□IL-2RA rs12569923, rs12722498 and IL-2RB rs3218264 polymorphisms might be related to CHD. The results required further validation by larger studies.

Introduction

Coronary heart disease (CHD), also known as coronary artery disease (CAD), is one of the leading causes of death worldwide. The disease is characterized by formation of arterial plaques which are mainly comprised of lipids, calcium and inflammatory cells(1) The pathogenesis of CHD is thought to be associated with multifactor, including atherosclerosis, obesity, hypertension, diabetes and smoking habits(2, 3). It was reported that inflammatory factors involving cytokines take an essential part in the progression of atherosclerosis, which eventually leads to CHD(4, 5). Although researches in CHD have been ongoing in the latest years, the specific mechanism remains to be further clarified. Notably, genetic and environmental factors are widely considered to play crucial role in the etiology of CHD, especially the genetic factors which are key to an individual's susceptibility to CHD, accounting for 30–60% of inter-individual variation in the risk of CHD(6, 7).

Interleukin-9 (IL-9) is a pleiotropic cytokine, and its gene is located on 5q31.1(8). Previous studies revealed that serum IL-9 level was significant higher in patients with atherosclerosis or CHD (9, 10). However, there were not any relative studies to research on the genetic effects of IL-9 with CHD. Additionally, Interleukin-2 (IL-2) was known as a T-cell growth factor when it was discovered in 1976 (11). The high affinity IL-2 receptor (IL-2R) is a heterotrimer consisting of the α chain (IL-2RA, CD25), the β chain (IL-2RB, CD122) and the common cytokine receptor γ chain (γc, CD132) (12). The previous studies indicated that IL-2, IL-2RA or IL-2RB genes caused muti-organ inflammation in both mice and human (13, 14). Importantly, IL-2RA and IL-2RB play crucial role in the development of CHD mainly through the combination with IL-2(15–17). It was reported that the gene variants in IL-2 were contributed the susceptibility to the CHD risk(18). However, it had little research to explore the IL-2R with CHD risk.

Therefore, in this study, we will conduct a case-control study to identify the association between CHD susceptibility and seven SNPs in the IL-9, IL-2RA and IL-2RB in the Chinese Han population. The study aims to identify a positive finding for the early prevention of CHD.

Materials And Methods

Study participants

This hospital-based case-control study was performed with 499 CHD patients and 496 healthy controls randomly recruited from the Second Affiliated Hospital of Hainan Medical University. All of the participants were genetically unrelated Chinese Han adults. The patients were diagnosed with CHD based on the coronary angiography or the criteria of typical clinical symptom, elevation of cardiac enzymes, and representative set of electrocardiogram(ECG) (19). The cases with severe anto-immunity disease such as systemic lupus erythematosus, inflammatory bowel disease, or Graves' disease were excluded. For controls, the healthy adults without any kinds of cardiovascular disease or relative medical history were recruited from the health checkup of the same hospital at the same period. We designed this protocol in compliance with the the Ethics Committee of the Second Affiliated Hospital of Hainan Medical University and the guidelines of the Declaration of Helsinki. All participants were provided and signed up the written informed consent.

Selection and genotyping of SNPs

We identified three single nucleotide polymorphisms (SNPs) in *IL-2RA*, three SNPs in *IL-2RB* and one in *IL-9* with a minor allele frequency (MAF) > 0.05 in Chinese Han population from NCBI dbSNP database (http://www.ncbi.nlm.nih.gov/projects/SNP) and the 1000 Genomes Projects (http://www.internationalgenome.org/). Fasting peripheral blood of all participants were collected in anticoagulant tubes and stored at -80°C. The whole blood genomic DNA extraction kit (GoldMag Co. Ltd, China) was used to extract DNA in accordance with manufacture's protocol, and the DNA content was measured by spectrometry (NanoDrop 2000 spectrophotometer, Thermo Scientific, USA). Agena MassARRAY Assay Design Software (version 3.0, Agena Bioscience, USA) was used to design multiplexed SNP MassEXTEND assay. And Agena MassARRAY RS100 was used to detect SNP genotyping(20, 21). Data were analyzed with Agena Typer Software (version 4.0, Agena Bioscience, USA).

Bioinformatics analysis

Online softwares, HaploReg v4.1 (https ://pubsbroad institute.org/mamma ls/haploreg/haploreg.php) and SNP info Web Server (https://snpin fo.niehs.nih.gov/snpinfo/index.html), took essential part in predicting the possible functional effects on these candidate SNPs.

Statistical analysis

SPSS software (version 20.0) was used for data analysis. The independent sample t-test or χ^2 test was used to examine the differences of basic parameters between the cases and controls. Hardy-Weinberg equilibrium (HWE) was tested by χ^2 test for each SNP selected in this study. The CHD risk associated with genotyping was estimated by odds ratios (ORs) with 95% confidence intervals (CIs) for five different genetic models. Multifactor dimensionality reduction (MDR) (version3.0.2) was performed to analyze the interactions between SNP and SNP in the CHD risk (22). The difference in clinical characteristics among different genotypes was analyzed using the one-way analysis and ANOVA test. For all test, a two-tailed *p*-value < 0.05 was considered statistically significant.

Results

Basic characteristic of the participants

The current study was included 499 CHD patients (319 males and 180 females) and 496 healthy controls (320 males and 273 females). The mean age of cases and controls were 61.34 ± 11.70 and 61.29 ± 8.94, respectively. Demographic and clinical characteristics were listed in **Table 1**, including age, gender, total cholesterol (TC), triglyceride (TG), high-density lipoprotein (HDL), low-density lipoprotein (LDL), platelet (PLT), plateletcrit (PCT), white blood cells (WBC), red blood cells (RBC), hemoglobin (HGB) and uric acid (UA). To examined the association in subgroup of CHD with diabetes or hypertension, the group of CHD patients were divided into four groups based on the presence or absence of hypertension or diabetes (CHD patients with diabetes or non-diabetes, CHD patients with hypertension or non-hypertension). And then we could observe that there were 59% CHD patients who were along with hypertension, while 20% CHD patients had diabetes.

Association of genetic polymorphism with the CHD risk

Basic information of the selected SNPs was presented in **Table 2**. All of genetic polymorphisms were complied with a Hardy-Weinberg equilibrium ($p > 0.05$). Significantly in **Table 3**, majority of the genetic model in *IL-9* (rs55692658) presented the increased risk with CHD (A vs G, OR = 1.72, 95% CI = 1.20-2.48, $p = 0.003$; GA vs AA, OR = 1.66, 95% CI = 1.13-2.42, $p = 0.010$; GG-GA vs AA, OR = 1.72, 95% CI = 1.17-2.51, $p = 0.006$; Log-additive, OR = 1.75, 95% CI = 1.20-2.53, $p = 0.003$). However, the SNPs in *IL-2RA* and *IL-2RB* showed no statistical significance with CHD risk (**Supplementary Table 1**).

Stratification analysis of SNPs with the CHD risk

Then we did stratified analysis of SNPs with CHD risk (**Table 4**). The results indicated that no matter in participants who were > 61 years or ≤ 61 years, *IL-9* rs55692658 and *IL-2RB* rs3218264 were significantly associated with the increased risk of CHD (In participants > 61 years old, *IL-9* rs55692658: G vs A, OR = 1.65, 95% CI = 1.03-2.65, $p = 0.037$, GG-GA vs AA, OR = 1.68, 95% CI = 1.01-2.81, $p = 0.049$, Log-additive, OR = 1.73, 95% CI = 1.05-2.83, $p = 0.030$; *IL-2RB* rs3218264: AG vs GG, OR = 1.61, 95% CI = 1.03-2.51, $p = 0.037$, AA-AG vs GG, OR = 1.59, 95% CI = 1.05-2.42, $p = 0.030$; In participants ≤ 61 years old, *IL-9* rs55692658: G vs A, OR = 1.90, 95% CI = 1.07-3.39, $p = 0.027$, GG-GA vs AA, OR = 2.00, 95% CI = 1.10-3.66, $p = 0.024$, Log-additive, OR = 2.00, 95% CI = 1.10-3.66, $p = 0.024$; *IL-2RB* rs3218264: AG vs GG, OR = 1.59, 95% CI = 1.03-2.46, $p = 0.036$, AG-AA vs GG, OR = 1.51, 95% CI = 1.01-2.28, $p = 0.046$) However, three selected SNPs in *IL-2RA* and another two SNPs in *IL-2RB* showed susceptibility to CHD risk with no statistical significance (**Supplementary table 2**).

By the stratification of gender shown in **Table 4**, we observed that *IL-9* rs55692658 was significantly correlated with the CHD risk (G vs A, OR = 2.32, 95% CI = 1.30-4.13, $p = 0.003$, GA vs AA, OR = 2.22, 95% CI = 1.21-4.09, $p = 0.010$, GG-GA vs AA, OR = 2.35, 95% CI = 1.28-4.30, $p = 0.006$, Log-additive, OR = 2.36, 95% CI = 1.31-4.25, $p = 0.004$). Nevertheless, *IL-2RA* rs12722498 presented a significantly decreasing-risk effect in female (G vs A, OR = 0.54, 95%CI = 0.30-0.96, $p = 0.033$, GA vs AA, OR = 0.52, 95% CI = 0.28-0.97, $p = 0.041$, GG-GA vs AA, OR = 0.51, 95% CI = 0.28-0.95, $p = 0.033$, Log-additive, OR = 0.54, 95% CI = 0.30-0.96, $p = 0.035$). On contrary, there were not any significant association between rs791588 and rs12569923 in *IL-2RA*, and three selected SNPs in *IL-2RB* and CHD risk (**Supplementary table 3**).

Association with hypertension and diabetes

To evaluate the association in subgroups of CHD with diabetes or hypertension, a total of 499 CHD patients was divided into four groups according to presenting or absenting hypertension or diabetes, respectively. The results shown in **Table 5** revealed that *IL-2RA* rs12569923 presented the increased risk with diabetes in CHD patients (G vs C, OR = 1.50, 95% CI = 1.04-2.17, $p = 0.028$; GG vs CC, OR = 2.70, 95% CI = 1.21-6.04, $p = 0.015$; GG vs GG-GC, OR = 2.59, 95% CI = 1.18-5.69, $p = 0.018$; Log-additive, OR = 1.43, 95% CI = 1.01-2.02, $p = 0.044$). On the contrary, all of the SNPs selected in this study were not significantly associated with hypertension in the CHD patients (**Supplementary table 3**).

Haplotype analysis with the risk of CHD

Furthermore, we researched the linkage disequilibrium (LD) and haplotype analyses of the *IL-2RA* polymorphisms. The reconstructed LD plot was presented in **Supplementary Figure 1**, and the LD block was comprised of two SNPs including *IL-2RA* rs12569923 and rs791588. The frequencies distribution of haplotypes in the case and control groups were shown in **Supplementary Table 4**. However, there was no significant association between haplotype and the risk of CHD.

SNP-SNP interactions

We used MDR analysis to assess the effect of SNP-SNP interaction among seven selected SNPs in *IL-9*, *IL-2RA* and *IL-2RB* (**Table 6**). In total, we found a three-locus mode including rs12569923 in *IL-2RA*, rs3218264 in *IL-2RB* and rs55692658 in *IL-9* was the best model (cross-validation consistency = 9/10, testing balanced accuracy = 0.539, $p = 0.002$). Obviously, there were interactions between locus and locus presented in a dendrogram and the Fruchterman-Reingold in **Figure 1** (A and B, respectively).

Genotypes and clinical characteristics

Additionally, we chose three SNPs in *IL-9*, *IL-2RA*, *IL-2RB* which presented the most significant association with the CHD risk according to the above studies to detected the relationship between different genotypes of these SNPs and clinical characteristics of patients, including total cholesterol (TC), triglyceride (TG), high-density lipoprotein (HDL), low-density lipoprotein (LDL), platelet (PLT), plateletcrit (PCT), white blood cells (WBC), red blood cells (RBC), hemoglobin (HGB), uric acid (UA). As shown in **Table 7**, there was no significant association between IL-9 rs55692658 and IL-2RA rs12569923 genotype polymorphisms and the clinical parameters mentioned above ($p > 0.05$). However, the AA genotype (1.61 ± 0.67 mmol/L) of *IL-2RB* rs3218264 were higher TG level than AG genotype (1.43 ± 0.60 mmol/L) and GG genotype (1.40 ± 0.64 mmol/L) ($p = 0.035$).

Discussion

CHD is considered to be a multifactorial disease, characterized by a chronic inflammatory process occurring primarily at the atherosclerotic plaque (23). Although the etiology of CHD remains to be further clarified, the genetic and environmental factors are regarded as important aspects in the progression of this disease. Previous studies have reported that serum interleukins level was correlated with the development of CHD, including IL-9 and IL-2 (23, 24). As a kind of key inflammatory factors, interleukins have been the center of attention up to now. Gradually, the multiple gene polymorphisms in interleukins with CHD risk began to be explore (25, 26), but little have reported about IL-9, IL-2RA and IL-2RB.

Therefore, we designed this case-control study to investigate the association between the SNPs in IL-9, IL-2A, IL-2RB and the susceptibility to CHD. The results revealed that there was a strong relationship of IL-9 rs55692658 with CHD risk. Furthermore, the stratification analysis by age showed that IL-9 rs55692658 and IL-2RB rs1573673 were significantly correlated with the increased risk of CHD without age relevant. For the subgroup of gender, we observed that IL-9 rs55692658 presented an increasing-risk effect, while IL-2RA rs12722498 was correlated with the decreased susceptibility to CHD in female. Moreover, IL-2RA rs12569923 was associated with the risk of diabetes in CHD patients. Since CHD represented a complex disease influenced by an interplay between genetic and environmental factors, SNP-SNP interaction studies might help identify the risk factors for CHD. Accordingly, we did MDR analysis to detected the potential SNP-SNP interactions in the selected SNPs. The results indicated that there was a strong interaction between IL-9 rs55692658, IL-2RA rs12569923 and IL-2RB rs3218264 regarding susceptibility to CHD. To our knowledge, this is the firstly to demonstrated the relationships between these SNPs in IL-9, IL-2RA, IL-2RB and the risk of CHD.

IL-9 gene was located on the long arm of chromosome 5. And IL-9 is specifically secreted not only by the eponymous Th9 cells, but also by a smaller amount by activated Th2 cells, Th17 cells, and regulatory T cells (27). Recently, few studies have revealed that IL-9 might mediate inflammatory cell infiltration into atherosclerotic lesions and might also play an important role in the atherosclerotic process (10, 28). According to these studies, we hypothesized that IL-9 SNPs might be related to CHD risk. In current study, we found that IL-9 rs55692658 was significant associated with an increased risk effect on CHD. Furthermore, gender and age were well-known risk factors in the prevalence of CHD (29). Therefore, we evaluated the correlation between the SNPs of IL-9 and CHD risk in different subgroups. Furthermore, by the stratification analysis with age and gender, we found that IL-9 rs55692658 contributed the susceptibility to CHD risk without age relevant. However, its increasing risk effect was significantly correlated with female.

Besides, serum levels of IL-2 was reported the relationship with CHD (16, 30). IL-2, a type 1 four α-helical bundle cytokine, was produced primarily by CD4 + T cells following their activation by antigen (31). IL-2R, especially the IL-2RA and IL-2RB which had high affinity to the IL-2, was obviously detected an increased serum level compared with the healthy controls according to the previous studies (31). Nevertheless, in the current study, we found that there was not any significant association between the selected SNPs in neither IL-2RA nor IL-2RB and the CHD risk. Thus, we did a further stratification analysis by age and gender. The results indicated that in patients who were at age ≤ 61, IL-2RB rs3218264 presented significantly increased risk effect on CHD. And in the subgroup of gender, it was revealed that IL-2RA rs12722498 was showed the decreased contribution to CHD susceptibility. The previous study had reported that CHD was associated with diabetes (32). Additionally, the morbidity and

mortality of CHD were related to degrees of increased blood pressure (33, 34). These suggested that CHD had close relationship with hypertension and diabetes. Hence, we did stratification analysis of hypertension and diabetes in CHD patients. The results significantly indicated that IL-2RA rs12569923 contributed to diabetes risk for individuals with CHD patients.

There are also limitations in the current study. At first, the sample size was not big enough and the subjects are limited to Chinese Han population. Consequently, selection bias is inevitable due to this study was hospital-based design. Then, CHD is a multifactorial disease with many other risk factors. We could not completely eliminate the potential influence of all the factors on the development of CHD. Hence, further studies with larger and multifarious sample are necessary.

Conclusion

In summary, the current study was the first to report that IL-9 rs55692658 significantly contributed the susceptibility to CHD risk. Specially, IL-2RA rs12722498 and IL-9 rs55692658 have significant differences in stratification analysis of gender. These results suggest that rs55692658 of IL-9 may serve as new biomarkers for the risk of CHD.

Declarations

Ethics approval and consent to participate

This study strictly obeyed the World Medical Association Declaration of Helsinki, which was also approved by the Ethical Committee of the Second Affiliated Hospital of Hainan Medical University. Written informed consent was obtained from each study participant.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Funding

Not applicable.

Author Contributions

XHC drafted the manuscript. XFW and ZZZ performed the DNA extraction and genotyping; YWC and XFW performed the data analysis; ZZZ and YWC performed the sample collection and information recording; XHC and CW conceived and supervised the study.

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Tables

Table 1 Characteristics of patients with CHD patients and healthy controls

Characteristics	Cases	Controls	*p*
	n = 499	n = 496	
Age (years) mean ± SD	61.34 ± 11.70	61.29 ± 8.94	0.939
Age (years)			
≤ 61	250 (50.0 %)	223 (55.0 %)	
61	249 (50.0 %)	273 (45.0%)	
Gender			
Male	319 (63.7%)	320 (64.5%)	0.795
Female	180 (36.3%)	176 (35.5%)	
TC (mmol/L)	4.09 ± 1.14	4.61 ± 0.94	**< 0.001**
TG (mmol/L)	1.46 ± 0.63	1.52 ± 0.68	0.252
HDL (mmol/L)	1.09 ± 0.28	1.08 ± 0.27	0.571
LDL (mmol/L)	1.92 ± 0.88	2.61 ± 0.78	**< 0.001**
PLT (10^9/L)	182.97 ± 59.56	210.33 ± 53.30	**< 0.001**
PCT (%)	0.02 ± 0.11	0.02 ± 0.07	0.878
WBC (10^9/L)	7.52 ± 3.03	5.83 ± 1.51	**< 0.001**
RBC (10^{12}/L)	4.85 ± 0.54	4.72 ± 0.71	**0.003**
HGB (g/L)	130.58 ± 29.82	145.53 ± 13.24	**< 0.001**
Urea (mmol/L)	5.52 ± 1.74	5.18 ± 1.31	**0.031**
UA(μmol/L)	286.88 ± 72.50	322.88 ± 66.86	**< 0.001**
Hypertension (yes/no)	295/204		
Diabetes (yes / no)	101/39		

TC, total cholesterol; TG, triglyceride; HDL, high-density lipoprotein; LDL, low-density lipoprotein; PLT, platelet; PCT, plateletcrit; WBC, white blood cells; RBC, red blood cells; HGB, hemoglobin; UA, uric acid

Variables are presented as the mean ± SD.

Bold values indicate significant difference ($p < 0.05$).

Table 2 Basic information and HWE about the selected SNPs

SNP ID	Genes	Chr: Position	Role	Alleles(A/B)	MAF		p-value for HWE	Haploreg 4.1	SNPinfo web server
					Cases	Controls			
rs55692658	IL-9	5: 135895540	intronic	A/G	0.082	0.049	0.623	Enhancer histone marks	TFBS
rs12569923	IL-2RA	10: 6042690	intronic	C/G	0.197	0.206	0.784	Enhancer histone marks, Motifs changed	
rs791588	IL-2RA	10: 6047379	intronic	G/T	0.363	0.374	0.774	Enhancer histone marks, Motifs changed	
rs12722498	IL-2RA	10: 6053873	intronic	C/T	0.079	0.088	0.405	Promoter histone marks, Enhancer histone marks, DNAse, Proteins bound, Motifs changed	
rs2281089	IL-2RB	22: 37136132	intronic	A/G	0.249	0.234	0.618	Enhancer histone marks	
rs3218264	IL-2RB	22: 37145958	intronic	C/T	0.485	0.486	0.073	Promoter histone marks, Enhancer histone marks, DNAse, Proteins bound, Motifs changed, Selected eQTL hits	
rs1573673	IL-2RB	22: 37172630	intronic	C/T	0.369	0.381	0.774	Enhancer histone marks, Motifs Changed, GRASP QTL hits	

SNP, single nucleotide polymorphism; CHD, coronary heart disease; MAF, minor allele frequency;

Table 3 Relationships between the SNPs of *IL-9* and CHD risk

SNP ID	Model	Genotype	Case	Control	Adjusted by age and gender	
					OR (95% CI)	*p*
IL-9 rs55692658	Allele	G	82	49	1.00	
		A	916	943	**1.72 (1.20-2.48)**	**0.003**
	Genotype	AA	420	447	1.00	
		GG	3	0	/	/
		GA	76	49	**1.66 (1.13-2.42)**	**0.010**
	Dominant	AA	420	447	1.00	
		GG-GA	82	132	**1.72 (1.17-2.51)**	**0.006**
	Recessive	GA-AA	496	496	1.00	-
		GG	3	0	-	
	Log-additive	-	-	-	**1.75 (1.20-2.53)**	**0.003**

SNP, single nucleotide polymorphism; OR, odds ratio; 95% CI, 95% confidence interval.

p values were calculated by logistic regression analysis with adjustments for age and gender.

"-" indicates Log-additive model; "/" indicates data missing.

Bold values indicate significant difference ($p < 0.05$).

Table 4 The SNPs of *IL-9, IL-2RA* or *IL-2RB* associated with CHD risk in the age and gender subgroup tests

SNP ID	Model	Allele/genotype	Case	Control	OR (95% CI)	*p*	Case	Control	OR (95% CI)	*p*
Age, years			**> 61**				**≤ 61**			
IL-9	Allele	A	453	515	1.00		463	428	1.00	
rs55692658		G	45	31	**1.65 (1.03-2.65)**	**0.037**	37	18	**1.90 (1.07-3.39)**	**0.027**
	Genotype	AA	207	242	1.00		213	205	1.00	
		GG	3	0	/	/	0	0	/	/
		GA	39	31	1.57 (0.93-2.65)	0.090	37	18	-	-
	Dominant	AA	207	242	1.00		213	205	1.00	
		GG-GA	42	31	**1.68 (1.01-2.81)**	**0.049**	37	18	**2.00 (1.10-3.66)**	**0.024**
	Recessive	GA-AA	246	273	1.00		250	223	1.00	0.477
		GG	3	0	/	/	0	0	/	/
	Log-additive	-	-	-	**1.73 (1.05-2.83)**	**0.030**	-	-	**2.00 (1.10-3.66)**	**0.024**
IL-2RB	Allele	G	237	283	1.00		253	247	1.00	
rs3218264		A	261	263	1.19 (0.93-1.51)	0.171	247	199	1.21 (0.94-1.57)	0.141
	Genotype	GG	55	78	1.00		60	73	1.00	
		AA	67	68	1.56 (0.94-2.58)	0.085	57	49	1.36 (0.81-2.28)	0.244
		AG	127	127	**1.61 (1.03-2.51)**	**0.037**	133	101	**1.59 (1.03-2.46)**	**0.036**
	Dominant	GG	55	78	1.00		60	73	1.00	
		AA-AG	194	195	**1.59 (1.05-2.42)**	**0.030**	190	150	**1.51 (1.01-2.28)**	**0.046**
	Recessive	AG-GG	182	205	1.00		193	174	1.00	
		AA	67	68	1.14 (0.76-1.71)	0.536	57	49	1.01 (0.65-1.57)	0.951
	Log-additive	-	-	-	1.24 (0.97-1.60)	0.090	-	-	1.02 (0.77-1.34)	0.894
SNP ID	Model	Allele/genotype	Case	Control	OR (95% CI)	*p*	Case	Control	OR (95% CI)	*p*
Gender			**Male**				**Female**			
IL-9	Allele	A	596	609	1.00		320	334	1.00	
rs55692658		G	42	31	1.38 (0.86-2.23)	0.180	40	180	**2.32 (1.30-4.13)**	**0.003**
	Genotype	AA	278	289	1.00		142	158	1.00	
		GG	1	0	/	/	2	0	/	/
		GA	40	31	1.34 (0.81-2.21)	0.248	36	18	**2.22 (1.21-4.09)**	**0.010**
	Dominant	AA	278	289	1.00		142	158	1.00	
		GG-GA	41	31	1.37 (0.84-2.26)	0.210	38	18	**2.35 (1.28-4.30)**	**0.006**
	Recessive	GA-AA	318	320	1.00		178	176	1.00	
		GG	1	0	/	/	2	0	/	/
	Log-additive	-	-	-	1.40 (0.86-2.28)	0.177	-	-	**2.36 (1.31-4.25)**	**0.004**
IL-2RA	Allele	A	579	582	1.00		340	312	1.00	
rs12722498		G	59	52	1.14 (0.77-1.69)	0.509	20	34	**0.54 (0.30-0.96)**	**0.033**
	Genotype	AA	262	268	1.00		161	141	1.00	
		GG	2	3	0.68 (0.11-4.10)	0.674	1	2	0.41 (0.04-4.67)	0.473
		GA	55	46	1.22 (0.80-1.88)	0.353	18	30	**0.52 (0.28-0.97)**	**0.041**
	Dominant	AA	262	268	1.00		161	141	1.00	
		GG-GA	57	49	1.19 (0.78-1.81)	0.413	19	32	**0.51 (0.28-0.95)**	**0.033**
	Recessive	GA-AA	317	314	1.00		179	171	1.00	
		GG	2	3	0.66 (0.11-3.97)	0.648	18	30	0.46 (0.04-5.22)	0.534
	Log-additive	-	-	-	1.14 (0.77-1.69)	0.508	-	-	**0.54 (0.30-0.96)**	**0.035**

OR, odds ratio; 95% CI, 95% confidence interval.

p values were calculated by χ^2 test with adjustment for age and gender.

"-" indicates Log-additive model; "/" indicates data missing.

Bold values indicate significant difference ($p < 0.05$).

Table 5 A list of SNPs associated with CHD in the subgroup tests (hypertension vs. non-hypertension and diabetes vs. non-diabetes)

SNP ID	Model	Allele/genotype	Case	Control	OR (95% CI)	*p*	Case	Control	OR (95% CI)	*p*
			Hypertension (Yes vs. No)				Diabetes (Yes vs. No)			
IL-2RA	Allele	C	464	337	1.00		151	650	1.00	
rs12569923		G	126	71	1.29 (0.93-1.78)	0.123	51	146	**1.50 (1.04-2.17)**	**0.028**
	Genotype	CC	187	144	1.00		61	270	1.00	
		GG	18	11	1.33 (0.60-2.94)	0.478	11	18	**2.70 (1.21-6.04)**	**0.015**
		GC	90	49	1.43 (0.95-2.17)	0.089	29	110	1.15 (0.70-1.89)	0.581
	Dominant	CC	187	144	1.00		61	270	1.00	
		GG-GC	47	32	1.42 (0.96-2.09)	0.079	40	128	1.37 (0.87-2.15)	0.177
	Recessive	GC-CC	277	193	1.00		90	380	1.00	
		GG	18	11	1.20 (0.55-2.62)	0.649	11	18	**2.59 (1.18-5.69)**	**0.018**
	Log-additive	-	-	-	1.28 (0.94-1.75)	0.117	-	-	**1.43 (1.01-2.02)**	**0.044**

SNP, single nucleotide polymorphism; CHD, coronary heart disease; OR, odds ratio; 95% CI, 95% confidence interval

p values were calculated by χ^2 test with adjustment for age and gender.

"-" indicates Log-additive model.

Bold values indicate significant difference ($p < 0.05$).

Table 6 MDR analysis of SNP-SNP interactions in relation to CHD risk.

Model	Training Bal. Acc	Testing Bal. Acc	OR (95% CI)	Testing χ^2 value	*p* value	CVC
rs3218264 (IL-2RB)	0.533	0.508	1.32 (1.01-1.71)	4.143	**0.042**	5/10
rs3218264 (IL-2RB), rs55692658 (IL-9)	0.551	0.539	1.52 (1.16-1.98)	9.384	**0.002**	9/10
rs12569923 (IL-2RA), rs3218264 (IL-2RB), rs55692658 (IL-9)	0.571	0.517	1.81 (1.37-2.38)	18.063	**< 0.0001**	5/10

MDR, multifactor dimensionality reduction; Bal. Acc., balanced accuracy; CVC, cross-validation consistency; OR, odds ratio; 95% CI, 95% confidence interval.

p values were calculated using χ^2 tests. $p < 0.05$ indicates statistical significance.

Table 7 Clinical characteristics of patients based on the genotypes of selected SNPs

Characteristics	*IL-9* rs55692658				*IL-2RA* rs12569923				*IL-2RB* rs3218264			
	AA	GA	GG	*p*	CC	GC	GG	*p*	AA	AG	GG	*p*
TC (mmol/L)	4.10 ± 1.17	4.02 ± 0.98	3.85 ± 0.21	0.832	4.08 ± 1.13	4.17 ± 1.14	3.77 ± 1.21	0.245	4.19 ± 1.12	4.04 ± 1.14	4.10 ±1.15	0.555
TG (mmol/L)	1.46 ± 0.63	1.52 ± 0.65	1.18 ± 0.25	0.618	1.48 ± 0.63	1.47± 0.63	1.27 ± 0.59	0.262	1.61 ± 0.67	1.43 0.60	1.40 ± 0.64	**0.035**
HDL (mmol/L)	1.10 ± 0.28	1.08 ± 0.25	1.15 ± 0.21	0.886	1.10 ± 0.28	1.11 ± 0.29	1.00 ± 0.02	0.184	1.10 ± 0.30	1.07 ± 0.24	1.13 ± 0.32	0.142
LDL (mmol/L)	1.92 ± 0.82	1.94 ± 1.18	1.53 ± 0.74	0.797	1.90 ± 0.89	1.98 ± 0.87	1.84 ± 0.87	0.616	1.91 ± 0.77	1.94 ± 0.97	1.89 ± 0.76	0.878
PLT (10^9/L)	183.55 ± 60.52	179.79 ± 55.27	185.00 ± 18.38	0.887	180.96 ± 59.58	174.29 ± 59.87	174.29 ± 59.87	0.293	189.67 ± 61.35	182.66 ± 59.42	177.99 ± 58.27	0.352
PCT (%)	0.02 ± 0.11	0.02 ± 0.08	/	0.927	0.02 ± 0.12	0.29 ± 0.78	/	0.806	/	0.03 ± 0.14	0.02 ± 0.07	0.419
WBC	7.57 ± 3.13	7.19 ± 2.45	8.59 ± 0.83	0.553	7.49 ± 3.02	7.66 ± 3.15	7.20 ± 2.50	0.734	7.79 ± 3.06	7.47 ± 3.10	7.40 ± 2.87	0.598
RBC	4.73 ± 0.66	4.67 ± 0.92	3.89 ± 0.16	0.194	4.74 ± 0.66	4.71 ± 0.70	4.59 ± 1.15	0.584	4.82 ± 0.63	4.69 ± 0.75	4.69 ± 0.68	0.284
HGB	131.16 ± 29.92	129.06 ± 27.92	93.00 ± 46.77	0.078	130.33 ± 29.43	131.80 ± 30.42	127.38 ± 32.07	0.764	127.0 ± 33.82	131.9 ± 29.03	130.8 ± 27.64	0.374
Urea	414 ± 5.41	72 ± 5.39	3 ± 7.23	0.190	5.34 ± 1.63	5.56 ± 1.84	5.59 ± 2.36	0.421	5.34 ± 1.67	5.37 ± 1.68	5.58 ±1.90	0.479
Uric acid (μmol/L)	287.0 ± 73.29	287.89 ± 69.00	247.67 ± 48.23	0.641	288.96 ± 71.71	282.91 ± 75.76	281.85 ± 66.91	0.687	77.3 ± 14.70	287.87 ± 72.59	287.50 ± 74.32	0.934

TC, total cholesterol; TG, triglyceride; HDL, high-density lipoprotein; LDL, low-density lipoprotein; PLT, platelet; PCT, plateletcrit; WBC, white blood cells; RBC, red blood cells; HGB, hemoglobin; UA, uric acid

"/" indicates data missing.

$p < 0.05$ indicates statistical significance

Figures

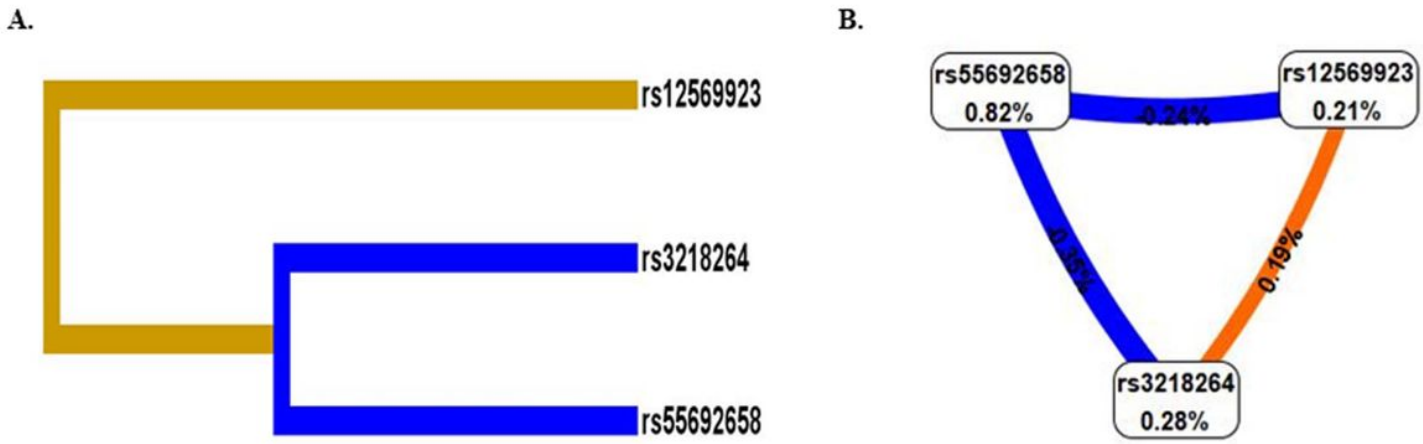

Figure 1