

Analysis for interaction between interleukin-35 gene polymorphisms and risk factors on susceptibility to coronary heart disease in the Chinese Han population

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Abstract

Objective

To explore the effect of single nucleotide polymorphisms (SNPs) of interleukin-35 (IL-35) gene and its relationship with environment on the risk of coronary heart disease (CHD).

Methods

Prior to the analysis, we performed Hardy Weinberg equilibrium test on the control group. The relationship between the four SNPs of IL-35 gene and the risk of coronary heart disease was studied by multivariate logistic regression. The best interaction was identified with generalized multifactor dimensionality reduction (GMDR). Logistic regression was used for investigation on association between four SNPs and CHD risk.

Results

Logistic regression analysis showed that the C allele of rs428253 and the G allele of rs2243115 were independently correlated with increased risk of CHD, and adjusted ORs (95%CI) were 1.91 (1.28–2.64) and 1.80 (1.30–2.23), respectively. However, there was no significant association between CHD and rs4740 or rs568408. GMDR model, indicated the best model for CHD risk consisted of rs428253 and current smoking, which scored 10/10 for both the sign test and cross-validation consistency ($P = 0.010$). Therefore, this overall multi-dimensional model had the highest cross-validation consistency, regardless of how the data were divided. This provided an evidence of gene-environment interaction effects. We also found that current smokers with rs428253 - GC/ CC genotype have the highest CHD risk, compared to subjects with never smokers with rs428253 - GG genotype, OR (95%CI) = 3.04 (1.71–4.41), after adjustment for age, gender, hypertension, T2DM and alcohol consumption status.

Conclusions

In this study, the C allele of rs428253 and the G allele of rs2243115, and the interaction rs428253 and current smoking were correlated with increased risk of CHD.

1. Introduction

In recent years, coronary heart disease (CHD) has become the main cause of the incidence rate and mortality rate in developed China and developing countries [1]. In recent years, the incidence rate and mortality rate of CHD in China has increased rapidly, and the age of onset is younger. Especially in young people (below 40 years old), about 700000 people die from CHD every year [2–3]. The etiology of CHD is very complex. The risk of CHD is not only affected by conventional environmental factors, but also by genetic variation [4]. Previous studies have reported many environmental risk factors related to coronary heart disease, including

blood lipid concentration, blood pressure, smoking, diabetes and so on [5]. In addition, inflammation also was reported associations with atherosclerosis development, which was an important risk factor for CHD [6].

Previous studies have reported that some inflammatory cytokine gene polymorphisms are associated with the risk of CHD, including cytokines in the IL-12 family [7, 8]. IL-35 is an anti-inflammatory cytokine and a member of IL-12 family. It is a heterodimer composed of p35 (IL-12A) and EBI3 subunits [9, 10]. Previous studies have reported that this genetic variation is related to the susceptibility of Alzheimer's disease and Graves' disease [11, 12], while ebi3 genetic variation may affect the risk of allergic rhinitis (AR) and tuberculosis in Chinese [13, 14]. Recently, only two studies [15, 16] have also studied the relationship between IL-35 gene polymorphism and CHD susceptibility. As far as we know, the relationship between IL-35 gene polymorphism and susceptibility to coronary heart disease has not been tested in the largest Han population in China. Although Lin et al [16] performed a case-control study for Chinese population, but these participants were all Chinese Zhuang. In addition, CHD is a multifactorial disease caused by both genetic and environmental factors [4] and gene-environment interactions [17]. The aim of this study was to evaluate the influence of SNPs within *IL-35* gene and their gene-environment interaction on susceptibility to CHD.

2. Methods

2.1 Participants

The population of current study was composed of 921 CHD patients and 926 age- and gender -matched controls. All CHD patients were recruited from the First Naval Hospital of Southern Theater Command. CHD was defined according to the World Health Organization criteria [18]. Participants with the following diseases will be excluded from the study cohort: heart related diseases, autoimmune diseases, chronic inflammatory diseases. The control participants were all the subjects who came to our hospital for routine occupational physical examination and voluntarily participated in this study. All the subjects were Han people, and there was no genetic relationship between them. Informed consent was obtained from each participant. This study has been approved by ethics committee of the First Naval Hospital of Southern Theater Command. In the study, we collected general demographic data and physical examination data of the subjects by questionnaire survey and physical measurement. The questionnaire includes general demographic information, life style, smoking and drinking.

2.2 Extraction of genomic DNA and genotyping

We selected 4 SNPs according to the following criteria from dbSNP algorithm (<http://www.ncbi.nlm.nih.gov/projects/SNP>): firstly, the MAF > 5% in the database; secondly, the relationship between SNPs and CHD was not verified in the previous studies. Genomic DNA from whole blood containing EDTA was isolated strictly following the instructions of the manufacturer. Four SNPs including rs428253, rs4740, rs2243115 and rs568408 were selected. Genotyping for rs2243115 and rs568408 was performed using polymerase chain reaction (PCR) and following restriction fragment length polymorphism (RFLP). Genotyping for rs428253 and rs4740 using TaqMan genotyping assays on an ABI Prism 7900HT Fast Real-Time PCR System according to the manufacturer's instructions (Applied Biosystems, Foster City, CA, USA). The primers and assays used for genotyping were listed in Table 1.

Table 1
Description of 4 SNPs and the assays or primers designed for genotyping

SNPs	Chromosome	Functional Consequence	Major/minor alleles	Genotyping assays or primers
EBI3 rs428253	19:4229916	Intron variant	G > C	GAATTTGAGTCACACTCATTCCCTTT[C/G] GTTTCTTTTTGGTTTTGTTTTTTGA
EBI3 rs4740	19:4236999	Missense variant, coding sequence variant	G > A	TGTGCGGCCCGAGCCAGGTACTAC[A/G] TCCAAGTGGCGGCTCAGGACCTCAC
IL-12A rs2243115	3:159988493	Upstream transcript variant, intron variant	T > G	Forward: 5'- AGAAAAGACCTGTGAACAAAACGACT - 3' Reverse: 5'- AGATGGCTCACTAGATGCCAGG - 3'
IL-12A rs568408	3:159995680	3 prime UTR variant, intron variant	G > A	Forward: 5'- GAAGGATGGGACYATTACATCCATAT - 3' Reverse: 5'- CAGGATGGATATTTTCCCTTCT - 3'

Statistical analysis

Hardy Weinberg equilibrium (HWE) test with SNPstats(<http://bioinfo.iconcologia.net/SNPstats>) Chi square test was used to compare the distribution of alleles and genotypes among groups. The mean \pm standard deviation (SD) was used to represent the continuous variables of normal distribution, and Student t test was used to compare the differences between the two groups. GMDR was used to determine the optimal interaction combination of four SNPs of IL-35 gene and environmental risk factors. Logistic regression was used to analyze the relationship between four SNPs and the risk of CHD. When p value is less than 0.05, the statistical significance is significant.

Results

In this study, a total of 1847 participants were enrolled, including 921 CHD patients and 926 controls. Table 2 showed the comparison results of different demographic characteristics between case group and control group. The mean age of all participants was (60.9 \pm 10.8) years old. The differences in males, BMI and age were not statistically significant between case and control group ($P > 0.05$). Compared with control group, the means of CRP levels, hypertension, T2DM of case group were significantly different (all $P < 0.05$). In addition, case group had a higher alcohol drinking and smoking rates compared with control group.

Table 2
General characteristics of study participants in CHD patients and controls

Variables	CHD patients (n = 921)	Controls (n = 926)	P values
Age (years) (means ± SD)	60.6 ± 11.3	61.3 ± 12.0	0.197
Males, N (%)	463 (50.3)	474 (51.2)	0.694
BMI (kg/m ²) (means ± SD)	24.6 ± 9.1	23.9 ± 9.5	0.106
CRP (mg/l) (means ± SD)	32.2 ± 16.7	12.9 ± 7.5	< 0.0001
Hypertension, N (%)	389 (42.2)	272 (29.4)	0.000001
T2DM, N (%)	170 (18.5)	104 (11.2)	0.000013
Smoking status, N (%)			0.000012
Current	267 (29.0)	187 (20.2)	
Never	654 (71.0)	739 (79.8)	
Alcohol drinking, N (%)			0.000089
Current	319 (34.6)	243(26.2)	
Never	602 (65.4)	683 (73.8)	
BMI: body mass index; CRP, C-reactive protein; T2DM: type 2 diabetic mellitus; SD: standard deviation			

All genotypes of the four SNPs followed Hardy-Weinberg equilibrium distribution in the control group (all P values were greater than 0.05). The C allele frequency of rs428253 gene was 20.5% in the normal control group and 30.2% in the CHD group. The G allele frequency of rs2243115 gene was 26.3% in patients with CHD and 17.8% in normal controls. Logistic regression analysis showed that the C allele of rs428253 and the G allele of rs2243115 were independently related to the increased risk of CHD. The adjusted OR (95% CI) was 1.91 (1.28–2.64) and 1.80 (1.30–2.23), respectively. However, there was no significant correlation between CHD and rs4740 or rs568408 (Table 3).

Table 3
Genetic risk evaluation of 4 SNPs within IL-35 gene and CHD risk

SNP	Genotypes or Alleles	Frequencies N (%)		Adjusted OR(95%CI)*	P values
		CHD cases (N = 921)	Controls (N = 926)		
EBI3- rs428253					
	GG genotype	458 (49.7)	594 (64.1)	1.00 (ref)	
	GC genotype	369 (40.1)	285 (30.8)	1.87 (1.21–2.58)	< 0.001
	CC genotype	94 (10.2)	47 (5.1)	2.06 (1.45–2.75)	< 0.001
	G allele	1285 (69.8)	1473 (79.5)	1.00	
	C allele	557 (30.2)	379 (20.5)	1.91 (1.28–2.64)	< 0.001
	P values for HWE		0.097		
EBI3- rs4740					
	GG genotype	483 (52.4)	543 (58.6)	1.00 (ref)	
	GA genotype	359 (39.0)	323 (34.9)	1.41 (0.95–1.97)	0.325
	AA genotype	79 (8.6)	60 (6.5)	1.56 (0.87–2.26)	0.561
	G allele	1325 (71.9)	1409 (76.1)	1.00	
	A allele	517 (28.1)	443 (23.9)	1.46 (0.92–2.04)	0.487
	P values for HWE		0.205		
IL-12A- rs2243115					
	TT genotype	513 (55.7)	633 (68.4)	1.00 (ref)	
	TG genotype	332 (36.1)	257 (27.8)	1.76 (1.32–2.18)	< 0.001
	GG genotype	76 (8.3)	36 (3.9)	1.93 (1.23–2.65)	< 0.001
	T allele	1358 (73.7)	1523 (82.2)	1.00	
	G allele	484 (26.3)	329 (17.8)	1.80 (1.30–2.23)	< 0.001
	P values for HWE		0.127		
IL-12A- rs568408					
	GG genotype	563 (61.1)	641 (69.2)	1.00 (ref)	
	GA genotype	312 (33.9)	253 (27.3)	1.31 (0.82–1.82)	0.436
	AA genotype	46 (5.0)	32 (3.5)	1.53 (0.74–2.33)	0.628

*Adjusted for gender, age, status of smoking and drinking and BMI.

SNP	Genotypes or Alleles	Frequencies N (%)		Adjusted OR(95%CI)*	P-values
		CHD cases (N = 921)	Controls (N = 926)		
	G allele	1438 (78.1)	1535 (82.9)	1.00	
	A allele	404 (21.9)	317 (17.1)	1.37 (0.80–1.91)	0.541
	P values for HWE		0.259		
*Adjusted for gender, age, status of smoking and drinking and BMI.					

Further association of the 4 SNPs with CHD was investigated using the GMDR model, and the cross-validation consistency and testing accuracy were calculated. With covariate adjustments for age, gender, BMI, hypertension, T2DM, alcohol drinking and smoking, the best model for CHD risk consisted of rs428253 and current smoking, which scored 10/10 for both the sign test and cross-validation consistency ($P = 0.010$, Table 4). Therefore, this overall multi-dimensional model had the highest cross-validation consistency. This provided an evidence of gene-environment interaction effects. In order to obtain the odds ratios and 95%CI for the joint effects of gene- smoking on CHD, we conducted stratified analysis for interaction effect using logistic regression. We found that current smokers with rs428253 - GC/ CC genotype have the highest CHD risk, compared to subjects with never smokers with rs428253 - GG genotype, OR (95%CI) = 3.04 (1.71–4.41), after adjustment for age, gender, hypertension, T2DM and alcohol consumption status. (Fig. 1)

Table 4
GMDR analysis for the best interaction combination models

Locus no.	Best combination	Cross-validation consistency	Testing balanced accuracy	<i>p-values</i> *
Gene- alcohol drinking interactions *				
2	1, 5	8/10	0.491	0.624
3	1, 2, 5	7/10	0.526	0.425
4	1, 2, 3, 5	5/10	0.518	0.746
5	1, 2, 3, 4, 5	6/10	0.521	0.857
Gene- smoking interactions **				
2	1, 6	10/10	0.632	0.010
3	1, 2, 6	7/10	0.532	0.172
4	1, 2, 3, 6	6/10	0.515	0.324
5	1, 2, 3, 4, 6	7/10	0.512	0.425
*Adjusted for age, gender, BMI, hypertension, T2DM and smoking.				
** Adjusted for age, gender, BMI, hypertension, T2DM and alcohol drinking.				
rs428253, rs4740, rs2243115, rs568408, current alcohol drinking and current smoking were symbolized as 1–6, respectively.				

Discussion

The current study indicated that both the C allele of rs428253 and the G allele of rs2243115 were correlated with increased risk of CHD, but rs4740 or rs568408 were not associated with CHD risk. The rs428253 belongs to *EBI3* gene, which located on chromosome 19p13.3. The rs2243115 belongs to IL-12A subunit, which is located on chromosome 3q25.33. Previously some studies have investigated the association between the two genes and inflammatory diseases [11, 12]. So far, there are few studies about the effect of IL-35 polymorphism on cardiovascular disease. Zhang et al confirmed that *ebi3*-rs428253 polymorphism is related to the risk reduction of chronic rhinosinusitis and allergic rhinitis [20]. So far, only two studies [15, 16] have confirmed the relationship between IL-35 gene and coronary heart disease risk, but the conclusions of these two studies are inconsistent. A study [15] for Mexico populations suggested that our study suggests a relationship of the *EBI3* SNPs with IL-35 levels, and *Ebi3*-rs428253 and *il-12a*-rs2243115 gene polymorphisms play an important role in the mechanism of CHD risk reduction. However, a recent case-control study in the Chinese population [16] suggested that there was a statistical correlation between the *EBI3* rs428253 mutation genotype and the risk of CHD, but there was no statistical significance between the mutation genotype of IL-12A-rs2243115 and the risk of CHD. The study also found that there was no significant difference in the level of IL-35 between different genotypes in the healthy control group. Although

two case-control studies have been performed previously, but the two studies concluded inconsistent results, and just one study was performed for Chinese Zhuang population, but no study focused on Chinese Han population, which was the largest nationality in China. So the difference in nationality for our study and previous studies may lead to different results. The biological mechanism of the association between IL-35 gene and coronary heart disease is not well established, but previous studies suggested that EBI3-rs428253 may be involved in the modification of LEF1 binding site [15, 22], and play an important role in granulocyte proliferation and differentiation [23]. SNP-rs428253 in EBI3 gene is related to the occurrence and development of CHD, which may be caused by regulating β -Catenin pathway and Treg pathway rather than by influencing the production of IL-35, because in the IL-35 levels was not different among different genotype of rs428253 in controls of study by Lin et al [16].

The pathogenesis of coronary heart disease is very complex. It is not only affected by genetic factors and environmental factors independently, but also by the synergistic effect between them [24, 25]. We were all known that alcohol drinking and smoking were two main modified risk factors for CHD [26, 27]. In this study, the smoking and alcohol drinking rates were higher in cases than in controls, which indicated that smoking and alcohol drinking were two risk factors for CHD. So we performed a GMDR analysis for gene-smoking or alcohol drinking interaction, and the results suggested a significant interaction between rs428253 and current smoking associated with CHD risk. Current smokers with rs428253 - GC/ CC genotype have the highest CHD risk, compared to subjects with never smokers with rs428253 - GG genotype. Environmental factors can cause phenotypic differences by influencing gene expression regulation. Therefore, the study of *IL-35* gene-environment interaction is helpful to better understand the occurrence of CHD.

There were several limitations in this study. Firstly, the sample size is not large enough, so this is only a preliminary study of the polymorphism of this locus, so the results obtained in current study need to be verified in the study with larger sample size and in different populations. Secondly, we just selected four loci in this study. In the future, we will study multiple loci of *IL-35* gene and CHD, in order to better understand the mechanism of CHD from the perspective of genetics. Lastly, the G allele frequency of rs2243115 was higher than that in gene database, so the selection bias may exist in this study.

The C allele of rs428253 and the G allele of rs2243115 were correlated with increased risk of CHD. We also found a significant interaction between rs428253 and current smoking associated with CHD risk. Although the previous studies have reported the relationship between the gene and CHD risk, but these studies concluded conflicting results, so the results obtained from this study verified this association. In addition, we also found the interaction between rs428253 and current smoking, which added more detailed mechanism for relationship between gene, environmental risk factors and CHD susceptibility.

List Of Abbreviations

CHD: coronary heart disease; SNP: single nucleotide polymorphisms; IL-35: Interleukin 35; GMDR: Generalized multifactor dimensionality reduction; PCR-RFLP: Polymerase Chain Reaction - based Restriction Fragment Length Polymorphism; HWE: Hardy–Weinberg equilibrium.

Declarations

Ethics approval and consent to participate:

this study has been approved by ethics committee of the First Naval Hospital of Southern Theater Command.

Consent for publication:

Not applicable

Availability of data and material:

Not applicable

Competing interests:

The authors declare that they have no competing interests

Funding:

Not applicable.

Authors' contributions:

Manuscript preparation, editing and review were conducted by HL and YXL; JYH participated in the interpretation of the studies and experiment; YFZ conducted study concepts and study design; guarantor of integrity of the entire study; data analysis and statistical analysis were conducted by KW.

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Figures

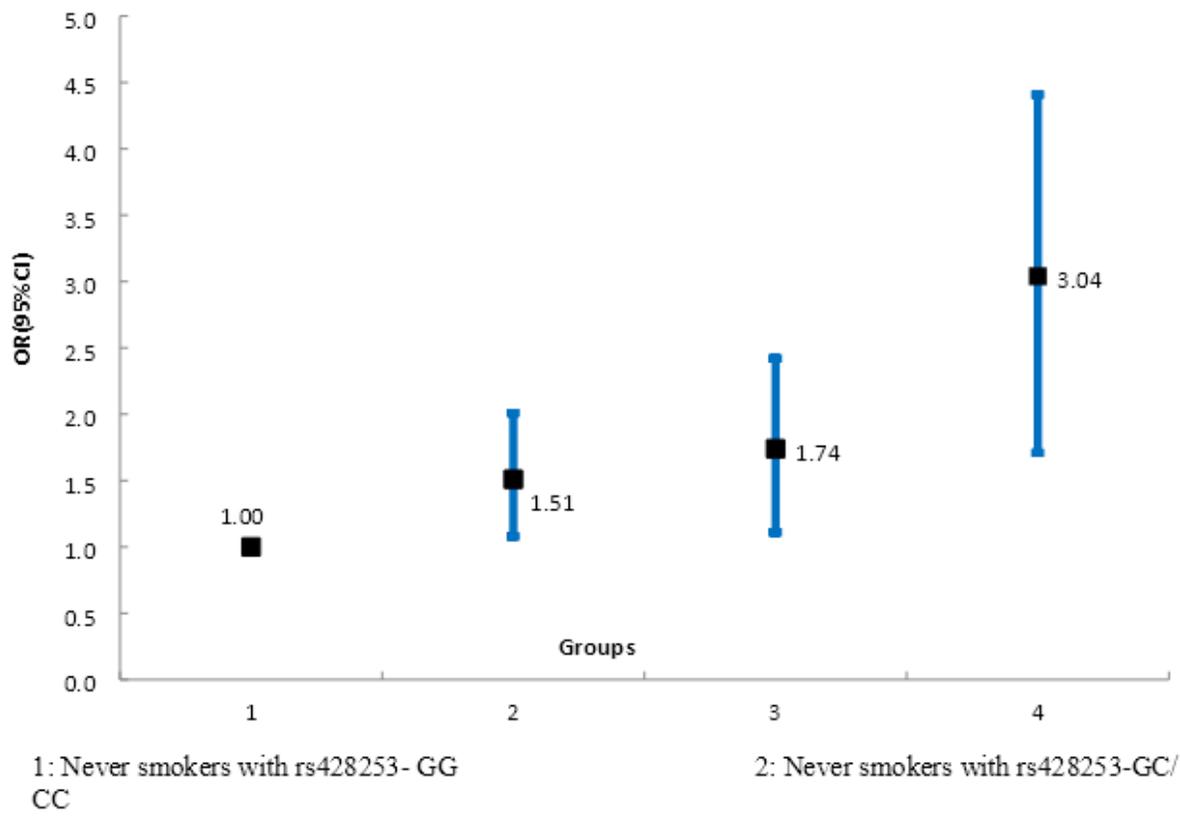


Figure 1

Stratified analysis for gene- smoking interaction on CHD risk using logistic regression