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Association of two variants in the interleukin-6 receptor gene and premature coronary heart disease in a Chinese Han population

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Abstract Two novel single nucleotide polymorphisms (SNPs; rs7529229 and rs2228145) in the interleukin-6 receptor (IL6R) gene have recently been associated with coronary heart disease (CHD) in a European population. We sought to replicate this finding and to investigate associations of these two SNPs with the severity and clinical phenotypes of premature CHD in a Chinese Han population. A total of 418 patients were studied, including 187 cases with coronary stenosis ≥50 % or acute myocardial infarction (males < 55 years and females < 65 -years) and 231 controls without documented CHD. A ligase detection reaction was performed to detect rs7529 229 and rs2228145. There were no differences between the controls and premature CHD groups in the frequencies for the three genotypes and alleles of rs7529229 and rs2228145 (all $P > 0.05$), nor did they differ between the two groups when grouped by gender (all $P > 0.05$). There were also no associations between these two SNPs and the severity of coronary lesions or clinical phenotypes of premature CHD (all $P > 0.05$). Our results do not support an association between rs7529229 or rs2228145 with premature CHD in the Chinese Han population. Further studies are warranted to elucidate the role of these two SNPs in the development of atherosclerosis and CHD.

Keywords Coronary heart disease · Single nucleotide polymorphisms · Gene · Interleukin-6 receptor

Introduction

Coronary heart disease (CHD) is one of the most common manifestations of atherosclerosis, and it is also one of the major causes of death in most countries including China [1]. CHD is regarded as a chronic inflammatory disease [2] and a link between CHD genetic susceptibility and the response to inflammatory signaling has been established [3].

Interleukin-6 (IL-6) is a multi-functional cytokine involved in various contradictory processes. It acts on a wide spectrum of target cells and exerts multiple functions during the immune response, hematopoiesis, neural differentiation, and the acute phase reaction [4]. It is considered one of the most important elements of the inflammatory reaction [5]. Also, IL-6 has been shown to inhibit lipoprotein lipase activity and stimulate lipolysis, which affects lipid profiles [6], contributing to the pathogenesis of atherosclerotic disease [7]. IL-6 acts via the membrane-bound or circulating soluble interleukin-6 receptor (sIL6R) on monocytes, hepatocytes, and endothelial cells [8]. sIL6R is composed of two subunits: an 80 kDa IL-6 binding protein (gp80) and a 130 kDa signal transducing protein (gp130) [9, 10]. Both gp80 and gp130 are expressed on the cell membrane or in functional soluble forms (sgp80 or sIL6R and sgp130) that may bind circulating IL-6 [11]. The IL-6–sIL6R complexes may affect cells that do not specifically express IL6R and that only present the ubiquitously expressed gp130.

Synthesis of IL-6 is stimulated by IL6R signaling. IL-6 binds to IL6R, and together they activate an intracellular signaling cascade leading to the inflammatory response

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[12]. High circulating concentrations of IL-6 are associated with an increased risk of CHD events in prospective observational studies [13–15]. IL6R signaling may be an important therapeutic target for prevention of CHD.

The IL-6R gene is located on human chromosome 1q21 [16], a region reported to be linked to dyslipidemia, metabolic syndrome, and type 2 diabetes [17–19]. In particular, IL-6R rs7529229 is a C/T variation in the IL6R gene (intronic) on human chromosome 1, with the polymorphism localized to a functional domain of the receptor protein. rs2228145 is a A/G/T variation in the IL6R gene (intronic) on human chromosome 1. This is a common polymorphism in IL6R marking a non-synonymous variant (Asp358Ala) with known functional consequences associated with differences in circulating concentrations of sIL6R, IL6, C-reactive protein, and fibrinogen [20]. Recently, two large case–control studies reported that rs7529229 and rs2228145 are strongly associated with CHD and cardiovascular events [20, 21].

Since genotype information is fixed, it is useful for early CHD risk prediction and individualized prevention if genetic risk markers are assessed and confirmed in different ancestries. In this study, we sought to replicate the association between rs7529229 and rs2228145 with the risk of CHD and to investigate associations between these two single nucleotide polymorphisms (SNPs) and the severity and clinical phenotypes of CHD in a middle-age Chinese Han population.

Methods

Subjects

The study cohort was comprised of 418 Chinese subjects, age 31 to 64 years (males < 55 years and females < 65 -years), consecutively enrolled to participate in this study for chest discomfort and suspectable CHD. Premature CHD was defined as clinical CHD occurring by age < 55 in males or < 65 in females [22] and was verified by coronary angiography (CAG). Premature CHD was present in 187 patients, 72 of whom were diagnosed with stable angina pectoris (SAP), 79 with unstable angina pectoris (UAP), and 36 with acute myocardial infarction (AMI). All patients having contraindications for heparin, auto-immunologic disease, congenital heart disease, syndrome X, severe kidney or liver disease, or malignant disease were excluded from this study. Smoking habits and family history of cardiovascular disease (CVD) were identified by the information obtained in the patients' questionnaires. The study was approved by the Ethics Committee of the Affiliated Zhongda Hospital of Southeast University, and all participants signed written informed consent. All patients came from the same geographical area with a similar socioeconomic and ethnic background.

Coronary angiography

All patients underwent elective CAG according to the Judkins technique. CHD was verified by angiography with $\geq$50 % luminal narrowing in at least one main coronary artery or a definite diagnosis of AMI defined using the WHO criteria. Gensini scores were calculated to reflect the extent of coronary lesions [23]. A total of 231 patients without detectable coronary stenosis were considered controls. The consensus of two cardiologists blinded to the study design was used to judge the grade of the coronary stenosis.

DNA extraction and genotyping

Peripheral venous blood was drawn from each participant. Genomic DNA was extracted using a QIAamp DNA Blood kit (Qiagen, Valencia, CA, USA). The SNPs rs7529229 and rs2228145 of the IL-6R gene were genotyped using a ligase detection reaction using TaqMan genotyping assays on an ABI Prism 377 Sequence Detection System (Applied Biosystems, Foster City, CA) according to the manufacturer's instructions. A successful genotyping rate of over 95 % was achieved for the entire SNPs test. Random duplicate samples and sequencing techniques were used for quality control in the genotyping, and the concordance was 100 %.

Statistical analysis

The statistical software package SPSS v.15.0 (SPSS, Chicago, IL, USA) was used for all statistical calculations. Continuous data were expressed as mean ± SD and the Student *t* test was employed to analyze differences between two study groups. Categorical variables were analyzed by χ^2-test. Age and body mass index (BMI, kg/m^2) were used as continuous variables. Hypertension, type 2 diabetes mellitus, smoking, family history of CVD, and gender were taken as categorical variables. Allele and genotype frequencies among CHD cases and controls were compared with values predicted by Hardy–Weinberg equilibrium using the χ^2-test. Distribution of genotypes at rs7529229 and rs2228145 among the different subgroups according to clinical phenotypes of premature CHD were determined by χ^2-test. For each odds ratio (OR), we calculated 95 % confidence intervals (CIs). Two-tailed *P* values <0.05 were considered significant.

Results

Basic characteristics of the study population

This study cohort included 187 patients with CHD and 231 controls. Table 1 summarizes the basic characteristics of the study population. No significant differences existed in baseline according to the composition of gender, ratio of hypertension, and mean values of BMI between premature CHD cases and controls. A higher prevalence of type 2 diabetes mellitus, smokers, and family history of CVD was found in patients with premature CHD when compared to controls.

Genotypes and allele distribution for rs7529229 and rs2228145 in patients with premature CHD and controls

There was no significant deviation from Hardy–Weinberg equilibrium in any of the samples studied. Of the 418 subjects participating in the study, analysis of CC, CT, and TT genotype frequencies of rs7529229 and CC, AC, and AA genotype frequencies of rs2228145 did not reveal any significant differences between the patients with premature CHD and controls (Table 2).

Table 2 also shows the evaluation of frequency of the polymorphisms according to gender. In the female group, the distributions of genotypes CC, CT, and TT of rs7529229 and genotypes CC, AC, and AA of rs2228145 did not differ significantly from that in the controls (all $P > 0.05$). Also, the distributions of the different alleles at rs7529229 and rs2228145 did not differ between female patients with premature CHD and female controls (all $P > 0.05$). There was also no significant difference in the distribution of the genotypes for these two SNPs between male patients with premature CHD and male controls (all $P > 0.05$) (data not shown).

Table 1 Baseline characteristics of the study population

	Controls	Premature CHD
Numbers (*n*)	231	187
Gender, male (%)	104 (45)	96 (51.3)
Age (years)	50.02 ± 6.37	51.26 ± 7.94
BMI (kg/m^2)	24.32 ± 4.79	25.65 ± 9.86
Hypertension, *n* (%)	129 (55.8)	109 (58.3)
Type 2 diabetes mellitus, *n* (%)	25 (10.8)	56 (29.9)†
Smokers, n (%)	60 (26)	92 (49.2)†
Family history of CVD (%)	70 (30.3)	78(41.7)*

Data are mean ± SD or number (%), as appropriate

* $P < 0.05$, † $P < 0.01$ vs. controls

BMI body mass index; *CHD* coronary heart disease; *CVD* cardiovascular disease

Association between SNPs rs7529229 and rs2228145 with the severity of coronary lesions and clinical phenotypes of premature CHD

We used Gensini scoring system to reflect the extent of coronary lesions and found that average Gensini scores were 58.46 ± 23.81, 57.52 ± 23.90, and 59.27 ± 25.01 in patients with CC, CT and TT genotypes at rs7529229 and 57.65 ± 24.76, 58.06 ± 23.81, and 58.68 ± 25.57 in patients with CC, AC and AA genotypes at rs2228145, respectively. There were no significant differences according to the Gensini scores among three genotypes in rs7529229 or rs2228145 (all $P > 0.05$).

With regard to clinical phenotypes, the patients with premature CHD were divided into three subgroups: SAP, UAP and AMI. Analysis indicated that no significant differences existed in the distribution of genotypes at rs7529229 or rs2228145 among the SAP, UAP, and AMI subgroups (all $P > 0.05$; Table 3).

Discussion

To our knowledge, this study represents the first report exploring the involvement of IL6R variants in CHD in a sample of patients from a Chinese Han population. However, our results do not support an involvement of SNPs rs7529229 or rs2228145 of the IL-6R gene in predisposition to premature CHD. The results of this study are inconsistent with the recent findings from large pooled samples from a European population [20, 21] in which these two SNPs were found to be associated with CHD.

Currently, the identification of novel genetic variants for use in assessing early risk of CAD is attracting increasing interest in medical labs throughout the world. Depending on the genetic information acquired, understanding disease etiology in terms of genetic determinants may be useful for identifying high-risk individuals and adapting therapeutic management to the individual's genetic make-up [24].

It was recently reported [21] that the rs7529229 variant was associated with reduced odds of CHD events, which suggests that targeting of IL6R could provide a novel therapeutic approach for the prevention of CHD. Although the IL6R rs7529229 variant was associated with reduced circulating C-reactive protein and fibrinogen concentrations, this observation should not be interpreted as an analysis indicating causality of C-reactive protein or fibrinogen in CHD. Furthermore, large Mendelian randomization studies using SNPs in the genes encoding

Table 2 Genotypes and allele distribution at rs7529229 and rs2228145 in premature CHD cases and controls

	Controls (*n*/%)	Premature CHD (*n*/%)	OR (95 % CI)	Female controls (*n*/%)	Female premature CHD (*n*/%)	OR (95 % CI)
rs7529229						
TT	76/32.9	62/33.2		34/26.8	27/29.7	
CT	105/45.5	85/45.4	1.02 (0.60–1.74)	63/49.6	44/48.4	1.19(0.55–2.54)
CC	50/21.6	40/21.4	1.01 (0.65–1.57)	30/23.6	20/21.9	1.05(0.52–2.07)
P	0.99			0.88		
T	257/55.6	209/55.9		131/51.6	98/53.8	
C	205/44.4	165/44.1	1.01 (0.77–1.33)	123/48.4	84/46.2	0.91 (0.62–1.33)
P	0.94			0.64		
rs2228145						
AA	75/32.5	57/30.5		33/26.0	25/27.5	
AC	105/45.4	90/48.1	0.97 (0.57–1.66)	64/50.4	46/50.5	1.13 (0.52–2.45)
CC	51/22.1	40/21.4	0.89 (0.57–1.38)	30/23.6	20/22.0	1.08 (0.54–2.13)
P	0.86			0.95		
A	255/55.2	204/54.5		130/51.2	96/52.7	
C	207/44.8	170/45.5	0.97 (0.71–1.28)	124/48.8	86/47.3	0.94 (0.64–1.37)
P	0.85			0.74		

The χ^2 test for genotypes and alleles and likelihood ratio test were used in the analysis. *n* number of individuals, with percentage of the total group in parenthesis. *CHD* coronary heart disease, *95 % CI* 95 % confidence interval, *OR* odds ratio

Table 3 Distribution of genotypes at rs7529229 and rs2228145 among three clinical phenotypes of premature CHD

	Clinical phenotypes of premature CHD			*P* value
	SAP	UAP	AMI	
rs7529229				
CC (*n*/%)	17/9.1	16/8.6	7/3.7	0.87
CT (*n*/%)	31/16.6	35/18.7	19/10.2	
TT (*n*/%)	24/12.8	28/15.0	10/5.3	
rs2228145				
CC (*n*/%)	19/10.2	14/7.5	7/3.7	0.63
AC (*n*/%)	30/16.0	41/21.9	19/10.2	
AA (*n*/%)	23/12.3	24/12.8	10/5.3	

AMI acute myocardial infarction, *CHD* coronary heart disease, *SAP* stable angina pectoris, *UAP* unstable angina pectoris

C-reactive protein and fibrinogen [25–28] have suggested that neither is a causal mediator of CHD.

The SNP rs2228145 (Asp358Ala) is the only known IL6R SNP that affects IL-6R protein function. In a recent study [21], rs2228145 was not shown to be associated with lipid concentrations, blood pressure, adiposity, dysglycaemia, or smoking. rs2228145 was associated with risk of CHD, though it is not related to IL6R mRNA levels or IL-6 production in monocytes. However, in our study, there existed comparable frequencies of genotypes and alleles of rs7529229 and rs2228145 between the patients with premature CHD and controls, and no association between rs7529229 or rs2228145 and a risk of premature CHD was found. Our study only enrolled middle-aged patients, and this differs from other studies [20, 21]. However, it is known that the results of studies exploring a genetic effect on CHD obtained in a young CHD group [29, 30] may be more reliable than that from an elderly or general group. This is because in older groups many other coexisting factors, including environmental factors, might have contributed more to the progress of CHD, thus complicating the analyses. The reason for the discrepancies between our study and other studies may partly be ethnicity-related factors contributing to the prevalence of genetic polymorphisms or gene-environment interactions.

In the present study, patients were subgrouped with regard to the genotypes at two SNPs, clinical phenotypes of premature CHD and gender, and our analysis did not show any association between the distribution of genotypes at rs7529229 or rs2228145 among any of these subgroups; nor did there exist any association between the severity of coronary lesions assessed by Gensini scores and three genotypes in rs7529229 or rs2228145.

Epidemiological data showed that the risk of CHD differs between female and male patients. This fact may be a result of exposure to different risk factors involved in the disease process and hormonal differences [31, 32] or other gender-specific effects [33]. In the present study, the distribution of genotype and allele frequencies at rs7529229 and rs2228145 did not differ between premature CHD and controls in either the male or female groups. Taken

together, the analyses presented in this current study may suggest that these two loci cannot explain either the susceptibility to premature CHD or the clinical phenotypes or severity of premature CHD. Thus, the available data do not consistently support that these two SNPs are involved in the genetics and/or the aetiology of CHD.

The present study has several strengths and limitations. First, all subjects underwent CAG, and this made the enrollment criteria more concise and accurate to test the true association between rs7529229 and rs2228145 with the risk of premature CHD. Second, the association between these two SNPs and premature CHD has not been previously studied in an Asian population and, thus, our study is novel and contributes new data to the body of knowledge in this field. This study presents some limitations. Because the studied cohort was obtained from a single hospital in China and all subjects were of the same ethnicity, the samples might not include all the characteristics of patients from other centers; thus, the results may not be generalized to other ethnic groups in which disparities in population composition or geographical and ethnic backgrounds may exist. Finally, as generally accepted, CHD is a disease influenced by multiple genes and environmental factors. As such, many other genes could be potential candidates influencing the occurence of premature CHD.

In conclusion, our results do not support an association between rs7529229 or rs2228145 and the occurrence of premature CHD in the Chinese Han population. Further studies are warranted to elucidate the role of these two SNPs of the IL-6R gene in the development of atherosclerosis and CHD.

Conflict of interest The authors declare that they have no competing interests.

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