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ORIGINAL ARTICLE

Coronary heart disease and chronic periodontitis: is polymorphism of interleukin-6 gene the common risk factor in a Chinese population?

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OBJECTIVES: Coronary heart disease (CHD) and chronic periodontitis (CP) both are multifactorial chronic diseases and related to inflammation. Interleukin-6 (IL-6) plays an important role in the pathogenesis of inflammatory diseases. The purpose of the study was to investigate the association among IL-6 gene polymorphisms, CP and CHD susceptibility in a Chinese population.

MATERIAL AND METHODS: The investigation was conducted as a case-control study involving 505 individuals: 113 patients with CHD and CP, 84 patients with CHD, 178 patients with CP and 130 control individuals. The polymorphisms of IL-6 gene were analyzed by polymerase chain reaction-restriction fragment length polymorphism. Relationships between the distributions of the genotypes and risk factors were also assessed.

RESULTS: Mutations at the loci -174 G/C, -597 G/A of IL-6 were rare in a Chinese population. No significant difference for IL-6-572C/G polymorphism was detected among moderate CP group, severe CP group and control ($P = 0.312$ and 0.481), significant differences were found between CHD groups and non-CHD groups ($P \leq 0.001$). After adjustment for CHD risk factors, the G allele resulted in an increased risk (OR = 1.676-1.856), the GG/CG genotype was nearly two times higher risk compared to CC genotype (OR = 2.010-2.136).

CONCLUSIONS: IL-6-572C/G polymorphism did not correlate with CP susceptibility, but might be a potential risk factor for CHD in a Chinese population.
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Keywords: chronic periodontitis; coronary heart disease; interleukin-6; gene polymorphism

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Introduction

Coronary heart disease (CHD) is a kind of multifactorial chronic diseases. In CHD, infections including of periodontal infection and other body infections are proposed to be risk factors (Dorn *et al*, 1999; Hujoel *et al*, 2001; O'Connor *et al*, 2001). Inflammation plays a central role in atherogenesis from endothelial cell expression of inflammatory cytokines, platelet adhesion and aggregation, smooth muscles cells and connected tissues proliferation to the development of the atherosclerosis (Alexander, 1994; Hansson, 2005). CP is an inflammatory disease and is the most frequently occurring form of infections in adults. Exposures to infections like periodontal diseases have been postulated to perpetuate inflammatory events in atherogenesis (David *et al*, 2007). In recent years, an increasing number of epidemiological studies have indicated that subjects with periodontitis may have increased risk of cardiovascular events, it was concluded that periodontal disease was modestly associated with atherosclerosis, myocardial infarction, and cardiovascular events (Beck *et al*, 1996; Hujoel *et al*, 2000; Scannapieco *et al*, 2003). Two mechanistic hypotheses have been proposed regarding the etiological pathways of the association between periodontitis, systemic inflammation and cardiovascular diseases: one focuses on the chronic infectious burden that periodontitis may represent for the organism via access of either microorganisms or endotoxins; the others see the diseased periodontium as a source of systemic inflammatory mediators. Systemic inflammation seems to be central for explaining the nature of the link between chronic infections and atherosclerosis (Danesh, 1999; Koenig, 2001; Libby, 2006; David *et al*, 2007).

CHD and CP both are considered as complex chronic diseases relating to multiple genes, and associated with inflammation (Lowe, 2001; David *et al*, 2007; Kullo and Ding, 2007; Yoshie *et al*, 2007). Thus, it is postulated that CP and CHD may share the common susceptibility genes. Identifying these genes can therefore result in novel diagnostics for risk assessment, early detection,

individualized treatment approaches for the two diseases especially for CHD.

So far, there is little information in the literatures about screening the common susceptibility genes for the two diseases. Polymorphisms of interleukin-1, interleukin-6, interleukin-10, tumor necrosis factor-alpha (TNF) have been reported in relation to CHD or CP, respectively (Kornman *et al*, 1997; Laine *et al*, 2001; Latkovskis *et al*, 2004; Arman *et al*, 2008; Manginas *et al*, 2008; Morange *et al*, 2008). Among these inflammatory cytokines, IL-6 is a circulating cytokine known to be secreted from a number of different cells including activated macrophages and lymphocytes in infections and injures. It regulates inflammatory reaction, accelerates bone resorption (Ishimi *et al*, 1990) and plays a key role in atherogenesis and thrombosis (Yudkin *et al*, 2000; Ikeda *et al*, 2001). IL-6 is elevated in the presence of systemic infection or inflammation. Increased level of IL-6 has been found in plasma and gingival crevicular fluid in patients with periodontitis (Geivelis *et al*, 1993). IL-6 also plays a key role in promoting atherogenesis and CHD (Vallance *et al*, 1997; Woods *et al*, 2000; Yudkin *et al*, 2000; Ikeda *et al*, 2001). Therefore, it was supposed that IL-6 genetic polymorphisms might represent a good candidate gene for susceptibility to CP and CHD. Three single nucleotide polymorphisms in IL-6 promoter (at loci-174G/C, -572C/G, -597G/A) were reported to influence IL-6 transcriptional regulation (Terry *et al*, 2000). The locus IL-6-174G/C genetic polymorphism was found in relation to CP or CHD susceptibility in Caucasian populations (Rauramaa *et al*, 2000; Trevilatto *et al*, 2003; Georges *et al*, 2001; Holla *et al*, 2004), IL-6-572G/C was reported to relate to cardiovascular risk events in a Chinese population (Wei *et al*, 2006), but very little literature had mentioned whether IL-6 gene polymorphisms (at loci-174G/C, -572C/G, -597G/A) directly increased the developing and severity of both periodontitis and CHD. Therefore, the aim of the study was designed to detect whether polymorphisms of interleukin-6 gene were associated with the susceptibility or severity of both CP and CHD in a Chinese population.

Materials and methods

Subjects

In a case-control design, 505 individuals were divided to four groups: 113 individuals with CHD and CP, 84 individuals merely with CHD, 178 individuals merely with CP and 130 control individuals without CHD and CP. The CHD patients were diagnosed at the Department of Cardiology, Zhujiang Hospital of Southern Medical University and the Second Hospital of Guangzhou Medical College. The CHD patients were divided to two groups: CHD group and CHD&CP group according to periodontal examination. CP patients (CP group) were recruited from the Department of Periodontology, Guangdong Provincial Stomatological Hospital, the Affiliated Stomatology Hospital of Southern Medical University. Subjects (control group) without CHD and CP were recruited from a community health checkup to match the age and gender distribution of CHD and CP patients as much as possible. Enrollment started in August 2007 and ended in September 2008. All participants were Chinese Han ethnicity. The patients received by the departments of Cardiology of the hospital mainly came from Guangdong province, the details of the study were explained to every registered patient and his/her attending physician, about 30% CHD patients and 10% CP patients at clinic were invited and did not want to participate in this study.

The CP was defined according to the criteria of the 1999 International World Workshop for a Classification of Periodontal Diseases and Conditions (Armitage, 1999). All clinical examinations were performed at dental clinic. The examinations were conducted by a single examiner. All participants had at least 20 natural teeth. Clinical periodontal parameters including probing depth (PD) and clinical attachment loss (CAL) were recorded at six sites for each tooth with a Williams probe. Periodontal diagnoses were mainly based on CAL from six index teeth (16, 11, 26, 36, 31 and 46; Wang *et al*, 2007; Xiao *et al*, 2009). In brief, the diagnosis of moderate CP was made if $\geq 30\%$ of selected sites had $3\text{ mm} \leq \text{CAL} < 5\text{ mm}$(or mean CAL $\geq$ 3-5 mm), severe CP was made if $\geq 30\%$ of selected sites had CAL ≥ 5 mm (or mean CAL ≥ 5 mm). Controls and CHD patients without CP were defined by PD < 3 mm or no obvious CAL. Patients with CAL 1-2 mm were excluded from controls. Because the age range of participants is 41-79 years and most of them were incorrect brushing, 1-2 mm CAL was found commonly in the aged people, it was difficult to identify 1-2 mm CAL due to CP or incorrect brushing. Patients with aggressive periodontitis were excluded.

All CHD patients had a medical examination. In general, patients were required to have coronary catheterization demonstrating significant CHD (at least a 50% stenosis in one major epicardial coronary vessel). Ischemic heart disease was diagnosed when there was a history of angina pectoris (clinical symptoms and changes in ECG typical of myocardial ischemia and/or elevated myocardial biochemical markers) or myocardial infarction.

Personal data including gender, age, height, weight, cigarette smoking habits, alcohol drinking habits, and medical history were obtained with a questionnaire interview conducted by a trained interviewer. Arterial hypertension was diagnosed when its presence was documented in medical records or if ≥ 2 readings of blood pressure were ≥ 18.6 kPa (systolic) or $\geq$12 kPa (diastolic). Hypercholesterolemia was defined as either a need for hypolipemic drugs or a total cholesterol level $> 5.2\text{ mmol l}^{-1}$, high low-density lipoprotein cholesterol ($> 100\text{ mg dl}^{-1}$), low high-density lipoprotein cholesterol ($< 40\text{ mg dl}^{-1}$), obesity was calculated by body mass index (BMI $\geq 25\text{ kg m}^{-2}$).

Participants with chronic inflammatory diseases, hepatitis, HIV infection, nephritis, pregnancy, diabetes mellitus, antibiotics taken in the previous 3 months were excluded.

This study was approved by the Ethics Committee of Guangdong Provincial Stomatological Hospital of

Southern Medical University, and an informed consent was obtained from each individual.

Sample collection and DNA extraction

Buccal swab samples were obtained from each subject. DNA was extracted from the buccal swab samples by the Chelex-100 resin method as previously described (Duan *et al*, 2001). Briefly, buccal swab samples were placed into 1.5 ml tubes containing 200 ml 5% Chelex-100 and 6 ml proteinase K (20 mg ml^{-1}) in sterile water. After vortexing, the tubes were incubated at 55°C for 3 h, boiled for 8 min, and placed on ice for 5 min, centrifugated at 12 000 *g* for 5 min at 4°C, approximately 200 ml supernatant containing DNA was collected, and pH was adjusted at 8.0 by the addition of 1 ml 100 TE buffer (1 M Tris–HCl, pH 8.0, 100 mM EDTA, pH 8.0). The DNA concentration of each sample was determined by ultraviolet spectrophotometry. The supernatant containing DNA was stored at −20°C for further analysis.

Genotyping

Genotyping of IL-6 -174G/C, -572C/G, -597G/A was performed by polymerase chain reaction (PCR) restriction fragment length polymorphism (PCR-RELP) method. The primers were as follows:

IL-6-174 (Rs1800795); *F*, 5′-tgacttcagctttactctttg t-3′; *R*: 5′-ctgattggaaaccttattaag-3′.

IL-6-597 (Rs1800797); *F*: 5′-ctcctctaagtgggctgaag-3′, *R*: 5′-caagcctgggattatgaaga-3′.

IL-6-572 (Rs1800796); *F*: 5′-gagacgccttgaagtaactg-3′, *R*: 5′-aaccaaagatgttctgaactga -3′.

The PCR was carried out in a 25 μl reaction mixture containing 100 ng genomic DNA, 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 1 mM each primer, reaction buffer, and 0.625 U Taq polymerase. The following cycling conditions were used: initial denaturation at 94°C for 3 min, followed by 35 cycles of 94°C for 30 s, 56°C for 30 s (52°C for 30 s for -174G/C), 72°C for 20 s, and a final extension of 72°C for 5 min. Ten microliters of the PCR products were digested respectively with NlaIII (-174G/C), BsrBI (-572C/G), FokI (-597G/A) overnight at 37°C (Fernandez-Real *et al*, 2000; Komatsu *et al*, 2005; Seow *et al*, 2005). The restriction products were electrophoresed in an 8% polyacrylamide gel (in 0.5 × TBE buffer, voltage 300 V, 30 min) and developed by a DNA silver staining method. The genotyping was performed by an experimenter blinded to sample identity.

To evaluate the consistency and stability of PCR-RELP, 10% samples were randomly duplicated tested. The PCR products of two or more samples in each genotype were selected to sequence to confirm the PCR-RELP results (Invitrogen, Shanghai, China).

Statistical analyses

All the analyses were performed with SPSS for window (Version 13.0, SPSS Inc., Chicago, IL, USA). The genotype distributions of the -174G/C, -597G/A, and -572C/G were tested for Hardy-Weinberg equilibrium. The continuous variables were compared with *t*-test and one-way ANOVA, the distributions of genotypes and allele frequencies among groups were compared using chi-square analysis and the multiple comparisons of genotypes and allele frequencies were performed by Chi-square test between each two groups. The differences in genotypes and allele frequencies between the CHD group and the non CHD group were further analyzed through Mantel-Haenszel statistic test with adjustment for CHD traditional risk factors (gender, age, smoking, drinking, hypertension, hyperlipidema, obesity). *P*-value < 0.05 was considered significant.

Results

The distributions of gender, age, genotype and allele frequencies of the -174G/C, -597G/A, and -572C/G were shown in Table 1. There were no significant

Table 1 Distribution of IL-6 genotypes and allele frequencies in four groups, *n* (%)

	Control (n = *130*)	*CP* (n = *178*)	*CHD* (n = *84*)	*CHD&CP* (n = *113*)	P-*value*
Age	52.3 ± 8.8	52.6 ± 7.8	52.1 ± 6.8	52.7 ± 6.4	0.731[a]
Gender (F/M)	54/76	93/85	39/45	62/51	0.080[b]
IL-6-572	130	178	84	113	
CC	95 (73.1)	120 (67.4)	42 (50.0)	64 (56.6)	0.016[b]
CG	32 (24.6)	52 (29.2)	38 (45.2)	43 (38.1)	
GG	3 (2.3)	6 (3.4)	4 (4.8)	6 (5.3)	
C	807 (79.9)	292 (82.0)	122 (72.6)	171 (75.7)	0.003[b]
G	203 (20.1)	64 (18.0)	46 (27.4)	55 (24.3)	
IL-6-597	130	178	84	83	
GG	127	204	91	83	
GA	0	0	0	0	
AA	0	0	0	0	
IL-6-174	130	178	84	90	
GG	129 (99.2)	177 (99.4)	84 (100)	89 (98.9)	0.818[b]
GC	1 (0.8)	1 (0.6)	0 (0)	1 (1.1)	
CC	0 (0)	0 (0)	0 (0)	0 (0)	
G	259 (99.6)	355 (99.7)	168 (100)	179 (99.4)	0.819[b]
C	1 (0.4)	1 (0.3)	0 (0)	1 (0.6)	

[a]*P*-value for age among four groups was obtained using *t*-test.
[b]*P*-value for gender, genotype distribution, allele frequencies among four groups was obtained using chi-square test.

differences among four groups in terms of age and gender ($P > 0.05$). The genotype distributions were in accordance with Hardy-Weinberg equilibrium (data not shown, $P > 0.05$). The prevalence of -174 variant C allele was very low (0-0.6%), there was no significant difference in genotypes or allele frequencies among four groups for the locus -174G/C ($P = 0.993$). For the locus -597G/A, only one wild-type homozygote was detected. For the locus -572C/G, there were significant differences in genotypes ($P = 0.016$) and allele frequencies ($P = 0.003$) among four groups.

For the locus -572C/G, further analysis of CP risk was shown in Table 2. No significant difference in genotypes or allele frequencies was detected among moderate CP group, severe CP group and control ($P > 0.05$), it indicated that IL-6-572C/G polymorphism was not significantly associated with susceptibility and severity of CP.

Multiple comparisons between two groups for IL-6-572C/G genotypes and allele frequencies were showed in Table 3. No significant difference in genotype or allele frequencies for IL-6-572C/G polymorphism was detected between CP group and Control group, CHD&CP group and CHD group, CHD&CP group and CP group ($P > 0.05$), but statistically significant differences were found between CHD&CP group and Control group, CHD group and CP group, CHD group and Control group ($P < 0.05$). To consider that distribution of genotypes or allele frequencies for the locus -572C/G had no significant effects on CP risk, therefore, CHD&CP and CHD group were combined to CHD groups (CHD&CP + CHD), CP and Control group were combined to non CHD groups (CP + Control), dramatic differences in genotypes and allele frequencies were shown between CHD groups and non CHD groups ($p \leq 0.001$), GG/CG genotype and G allele at the locus -572C/G were significantly higher in CHD groups and resulted in an increased risk ($OR_{GG+CG/CC} = 1.985$; 95% CI: 1.370-2.876, $OR_{G/C} = 1.737$; 95% CI: 1.274-2.369).

Several factors such as gender, age, smoking, drinking, hypertension, hyperlipidema, obesity were CHD traditional risk factors (Poulter, 1999). For the purpose of assessing CHD traditional risk factors on distributions of IL-6-572C/G genotypes and allele frequencies, age and gender had been balanced between the CHD group and the non CHD group in this case–control design, so we further assessed whether there existed differences in genotypes and allele frequencies distributions after adjusting for other CHD traditional risk factors (smoking, drinking, hypertension, hyperlipidema and obesity) (Table 4). For participants without smoking, hypertension, hyperlipidema, alcohol drinking and obesity (BMI < 25), the G allele was more frequent in patients with CHD than in non CHD controls ($P < 0.05$). the G allele resulted in an increased risk (OR = 1.676-1.856), the GG/GC genotype was more prevalent in CHD patients compared to in non-CHD participants ($P < 0.05$), the GG/CG genotype was nearly two times higher risk compared to CC genotype (OR = 2.010-2.136). For participants with smoking, hypertension, hyperlipidema, alcohol drinking and obesity (BMI ≥ 25), IL-6-572C/G polymorphism did not show any difference between CHD patients and non-CHD participants ($P > 0.05$). It was demonstrated that IL-6-572C/G polymorphism was significantly associated with CHD in the absence of CHD traditional risk factors such as smoking, hypertension, hyperlipidema, drinking and obesity.

Table 2 Distribution of IL-6-572C/G genotypes and allele frequencies in CP and control group

Variable	*Control* (n = *130*)	*Moderate CP* (n = *101*)	*Severe CP* (n = *77*)	P-*value*
PD (mm, mean ± SD)	1.68 ± 0.44	2.84 ± 0.62	3.96 ± 1.10	0.000[a]
				0.000[a]
CAL (mm, mean ± sd)	0.02 ± 0.11	3.71 ± 0.70	5.98 ± 0.84	0.312[b]
SNP (*n*, %)				
CC	>95 (73.1)	65 (64.4)	55 (71.4)	0.481[c]
CG	32 (24.6)	34 (33.7)	18 (23.4)	
GG	3 (2.3)	2 (2.0)	4 (5.2)	
C	222 (85.4)	164 (81.2)	128 (83.1)	
G	38 (14.6)	38 (18.8)	26 (16.9)	

[a]*P*-value was obtained using one-way ANOVA.
[b]*P*-value for genotype distribution among three groups was obtained using chi-square test.
[c]*P*-value for allele frequencies among three groups was obtained using chi-square test.

Table 3 Multiple comparisons between two groups for IL-6-572C/G genotypes and allele frequencies ($p\ \chi^{-2}$)

	CHD&CP vs *CHD*	*CHD&CP* vs *CP*	*CHD&CP* vs *control*	*CHD* vs *CP*	*CHD* vs *control*	*CP* vs *control*	*CP* + *control* vs *CHD&CP* + *CHD*
GG + CG	0.598/1.028	0.169/3.555	0.023/7.505	0.025/7.355	0.003/11.819	0.544/1.218	0.001/13.367
vs CC	0.560/0.469	0.073/3.436	0.007/7.383	0.016/6.085	0.001/10.543	0.275/1.229	0.000/12.326
G *vs* C							

Table 4 Analysis for CHD risk factors on distribution of IL-6-572C/G genotypes and allele frequencies

Risk factors	*Genotype/allele*	P-*value*[a]	*OR*	*95% CI*
Smoking				
No	GG + GC/CC	0.003	2.120	1.372-3.275
	G/C	0.002		
Yes	GG + GC/CC	0.268	1.785	1.241-2.568
	G/C	0.127		
Drinking				
No	GG + GC/CC	0.002	2.010	1.351-2.990
	G/C	0.001		
Yes	GG + GC/CC	0.304	1.788	1.282-2.493
	G/C	0.619		
Hypertension				
No	GG + GC/CC	0.025	2.055	1.194-3.537
	G/C	0.008		
Yes	GG + GC/CC	0.890	1.853	1.183-2.901
	G/C	0.880		
Hyperlipidema				
No	GG + GC/CC	0.002	2.136	1.402-3.256
	G/C	0.001		
Yes	GG + GC/CC	0.568	1.856	1.312-2.625
	G/C	0.446		
BMI				
< 25	GG + GC/CC	0.009	2.032	1.216-3.327
	G/C	0.011		
≥ 25	GG + GC/CC	0.050	1.676	1.137-2.470
	G/C	0.028		

[a]*P*-value for genotype and allele distributions between CHD groups and non CHD groups was obtained using chi-square test.

Discussion

To our knowledge, there was little literature about the association among IL-6 genetic polymorphisms, CP and CHD. The following IL-6 genetic polymorphisms have been reported respectively in relation to periodontitis or CHD: IL-6-174G/C was found in relation to CP susceptibility in a Caucasian Brazilian population (Trevilatto *et al*, 2003). The Czech study reported that the IL-6-572C/G polymorphism may be one of protective factors associated with lower susceptibility to CP (Holla *et al*, 2004). The Poland research showed that the highest IL-6 serum levels were found in the -174GG and the lowest in the -174CC genotype patients, IL-6 polymorphisms corresponded to the number of severely stenosed coronary arteries (Mysliwska *et al*, 2006). Antonicelli *et al* (2005) found that IL-6-174G/C polymorphism is an independent predictor of cardiovascular death after an acute coronary syndrome in Italian male patients. Otherwise, In contrast to these conclusions, Basso *et al* (2002) reported that -174CC genotype was associated with a lower risk of CHD, the -572C/G polymorphism was not significantly associated with CHD risk. Sie's meta-analysis did not show a significant association between the IL-6-174 genotype and risk of CHD (Sie *et al*, 2006). These contradictory conclusions could be explained as different ethnic background, regions, sample size, diagnose criteria and so on (Yoshie *et al*, 2007).

In this study, the variant allele frequencies of -174G/C and -597G/A were very low. In 533 samples, for -174G/C, only 4 GC heterozygotes were found, others are GG homozygotes, and for -597G/A, the variant allele A was not detected. The -174G/C and -597G/A loci may be non-polymorphic in a Chinese population. Our findings agreed with several East-Asian studies that IL-6-174G/C, IL-6-597G/A polymorphisms might be useless in disease susceptibility markers in East-Asian populations (Zhai *et al*, 2001; Lira *et al*, 2002; Chung *et al*, 2003; Park *et al*, 2003). On the other hand, the frequency of G allele of -572C/G was 0.18-0.27, IL-6-572C/G polymorphism may be useful in disease susceptibility markers in Chinese. In our results, no significant difference in genotype or allele frequencies for IL-6-572C/G polymorphism was detected among moderate CP group, severe CP group and control, but statistically significant difference was found between CHD groups and non CHD groups. GG/CG genotype and G allele at the locus -572 C/G resulted in an increased risk (OR = 1.985, $OR_{G/C}$ = 1.737). Thus, IL-6-572C/G polymorphism was associated with susceptibility to CHD, but not CP in a Chinese population. It would be explained that in multifactorial diseases, such as CHD, CP, diabetes, *et al* an individual may have increased susceptibility to severe disease due to different factors. For instance, one patient may have increased susceptibility due to smoking, another due to the IL-1 or other inflammatory cytokines genotypes, another due to, hypertension, hyperlipidema, poorly controlled diabetes (Armitage *et al*, 2000).

Several risk factors such as smoking, drinking, hypertension, hyperlipidema, obesity have already been identified in the etiology of atherosclerosis and CHD (Poulter, 1999). In this study, it was interesting to note that the -572 variant allele frequencies did not result in any statistically significant differences between CHD groups and non CHD groups when these factors were considered. After adjustment for above risk factors, IL-6-572C/G gene polymorphism was significantly associated with CHD. The results showed that IL-6-572C/G gene polymorphism might be a potential or weak risk factor for CHD in a Chinese population. It also indicated that CHD traditional risk factors such as smoking, hypertension, *et al*, may explain a part of CHD, other risk factors such as disease susceptibility genes are probably implicated in the CHD pathological process.

IL-6 is a powerful inducer of the hepatic acute phase response (Harris *et al*, 1999). Elevated concentrations of acute phase reactants, such as C-reactive protein (CRP), are found in patients with acute coronary syndromes, and predict future risk in apparently healthy subjects (Heinrich *et al*, 1990; Ridker *et al*, 2000). Higher plasma concentrations of IL-6 and CRP were observed for patients with the CG genotypes of the -572C/G polymorphism in a Sweden study (Malarstig *et al*, 2007). In some prospective studies of patients with CHD, there were weak associations between IL-6 polymorphisms and plasma concentrations of IL-6 and CRP (Brull *et al*, 2001; Bennermo *et al*, 2004; Latkovskis *et al*, 2004). Furthermore, IL-6 decreases lipoprotein lipase (LPL) activity and monomeric LPL levels in plasma, which

increases macrophage uptake of lipids (Van Snick, 1990; Greenberg *et al*, 1992). The level of plasma lipid in -572G allele carriers was significantly higher than non carriers (Wei *et al*, 2006). It indicated the possibility that IL-6-572C/G gene polymorphism seemed to affect CHD through elevation of plasma IL-6, CRP, lipid and lipoprotein. Anyway, it was shortcoming that the plasma IL-6, CRP, lipid and lipoprotein were not measured in this study, the influences of IL-6-572C/G gene polymorphisms on the levels of plasma IL-6, CRP, lipid and lipoprotein need to be further researched. Otherwise, it was necessary to collect more enough patients to analyze the association of the IL-6-572 genotype and CHD clinical status further.

In conclusion, Mutations at the loci -174 -572, -597 of IL-6 did not correlate with CP susceptibility, but IL-6-572C/G polymorphism was associated with susceptibility to CHD after adjustment for other risk factors such as smoking, hypertension, hyperlipidema, obesity and alcohol drinking. IL-6-572C/G polymorphism might be a potential risk factor for CHD in a Chinese population.

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Author contributions

The corresponding author of the manuscript is Prof. JinCai Zhang, who assumes the responsibility for the study and contributes to the research design and to revising the article critically. Dr Weihua Fan is responsible for analyzing data, and writing the manuscript. Dr LiMin Xiao is responsible for genotyping. DaLie liu, ChengJie Xie, and ShuYu Sun contribute to collecting sample.

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