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Original Article

Interleukin-10 promotor -592A/C polymorphism is associated with slow coronary flow in Han Chinese

Gui-Liang Shi[1*], Xing-Xing Cai[2*], Ya-Min Su[2], Chu Chen[2], Xin-Tao Deng[1], Hai-Yan Pan[2], Meng-Kan Fan[1], Jian-Hua Zhu[1], Min Pan[1]

[1]Department of Cardiology, Xinghua People's Hospital, Xinghua 225700, P. R. China; [2]Department of Cardiology, Affiliated Hospital of Nantong University, Nantong 226001, P. R. China. [*]Co-first authors.

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Abstract: An accumulating body of evidence suggests that slow coronary flow (SCF) phenomenon seems to be an early-form of atherosclerosis and low-grade inflammation plays a major role in the atherosclerotic vascular processes. Interleukin (IL)-10 is a multifunctional cytokine involved in both innate and adaptive immune response. The aim of the present study is to investigate the association of IL-10 gene -592A/C polymorphism with SCF in Han Chinese. 250 patients who underwent coronary angiography and had angiographically normal coronary arteries of varying coronary flow rates without any atherosclerotic lesion were enrolled in this study. Patients who had thrombolysis in myocardial infarction frame counts (TFC) above the normal cutoffs were considered to have SCF and those within normal limits were considered to have normal coronary flow (NCF). The PCR-based restriction fragment length polymorphism (PCR-RFLP) technique was used to assess the genotypes frequencies. The distribution of the IL-10 -592A/C genotypes (AA, AC, and CC) was 46.34%, 41.46%, and 12.20% in the NCF group, and 66.51%, 28.71%, and 4.78% in SCF subjects, respectively (P = 0.0280). The frequency of the A allele in the SCF group was significantly higher than that in the NCF group (80.86% vs. 67.07%, P = 0.0054). Compared with the CC genotype, the AA genotype had increased risk of SCF in both unadjusted and adjusted analyses. In SCF patients, the average serum IL-10 levels in AA genotype were statistically lower than in AC + CC genotype (P = 0.0000). These findings suggest that IL-10 -592A/C polymorphism is associated with SCF and the A allele has increased risk for SCF in Han Chinese.

Keywords: Slow coronary flow, interleukin-10, genetic polymorphism, coronary artery disease, Chinese

Introduction

Slow coronary flow (SCF) is an angiographic observation characterized by normal or near-normal coronary arteries with delayed opacification of the distal vasculature [1-3]. Since first description of this phenomenon by Tambe et al [1] in 1972, the prevalence of SCF is about 1% in patients undergoing coronary angiography for the suspicion of coronary artery disease (CAD) [4]. Micro- and macrovascular disease findings have been identified, such as hyperplastic fibromuscular thickening, myofibrillar hypertrophy, luminal narrowing, myofibrillar degeneration, endothelial degeneration, endothelial dysfunction and diffuse atherosclerosis [5]. However, the precise pathophysiological mechanisms and the clinical importance of SCF remain unclear at the moment.

IL-10 is a multifunctional anti-inflammatory cytokine that downregulates cell-mediated immune responses and cytotoxic inflammatory responses [6]. Interleukin-10 inhibits the production of pro-inflammatory cytokines by inhibition of T-helper 1 (Th1) lymphocytes and stimulation of B lymphocytes and Th2 lymphocytes and thus downregulates the inflammatory response [7]. As an inflammatory cytokine, IL-10 participates in the development of various diseases, such as chronic infection, kidney disease, cancer, and cardiovascular disease [8]. The gene encoding IL-10 is located on chromosome 1 (1q31-1q32). Three functional promoter single nucleotide polymorphisms (SNPs) in the IL-10 locus at -1082 (A to G, rs1800896), -819 (C to T, rs1800871), and -592 (A to C, rs1800872) from the transcriptional start site have been confirmed, and -819C/T is in tight

linkage disequilibrium with -592A/C [9, 10]. However, the -1082G allele is extremely rare in Chinese Han population [10, 11].

An accumulating body of evidence suggests that SCF phenomenon seems to be an early-form of atherosclerosis and low-grade inflammation plays a major role in the atherosclerotic vascular processes [12-14]. We hypothesized that IL-10 mediated inflammation may also be involved in SCF as well. Thus, we carried out a case-control study of IL-10 gene -592A/C polymorphism to evaluate its putative association with SCF in Han Chinese.

Subjects and methods

Study subject

A total of 250 consecutive patients who complained of chest pain or angina equivalent symptoms but had angiographically normal coronary arteries (NCA) of varying coronary flow rates without any atherosclerotic lesion were enrolled at the Affiliated Hospital of Nantong University. Details of medical history, as well as physical and laboratory examination, were obtained from all patients before performing the coronary angiography. Current smoking, hypertension, diabetes mellitus, and atrial fibrillation were defined according to past literature reports [3, 10, 15-18]. Patients with acute coronary syndrome, atrial fibrillation, hypertrophic cardiomyopathy, sinus node dysfunction or conduction disturbance, significant valvular disease, left ventricular dysfunction (ejection fraction < 50%), and neoplastic, renal, liver, or thyroid diseases were excluded. The study has been approved by the local medical ethics committee and written informed consent was obtained from all participants prior to the study.

Selective coronary angiography and determination of SCF

Selective coronary angiography was performed by radial approach using the standard Judkin's technique. Coronary angiograms were recorded in right and left oblique planes using cranial and caudal angulations, with a rate of 25 frames/s. Iopromide (Ultravist 370, Bayer, Guangzhou, China) was used as the contrast agent during coronary angiography in all participants. The coronary flow rates of all subjects were assessed using the thrombosis in myocardial infarction (TIMI) frame count (TFC) method because TFC is a simple, objective, reproducible, and quantitative index of coronary flow velocity [19]. The first frame used for counting was the first frame in which contrast fully entered the artery. The last frame counted was the one in which contrast first entered the end point branch off the target artery. The following distal landmark branches of the target artery were used for the analysis: the distal bifurcation in the left anterior descending artery (LAD), the distal bifurcation of the segment with the longest total distance in the left circumflex artery (LCX), and the first branch of the posterolateral artery in the right coronary artery (RCA). Because the length of the LAD is anatomically longer than that of the LCX and RCA, the LAD frame counts were corrected by dividing by 1.7 to obtain a corrected TFC, as previously reported [19]. The mean TFC for each SCF patient and normal coronary flow (NCF) subject was calculated by adding the TFC of the LAD, LCX, and RCA and then dividing the value obtained by 3. The mean TFC reported by the two independent observers were compared to assess interobserver reliability. A discrepancy was subsequently resolved by a third observer. SCF was diagnosed when mean TFC was more than 27 [19].

Biochemical analysis

Venous blood samples were obtained after at least a 10-hour overnight fast and then centrifuged at 2500 rpm for 30 minutes at 4°C and immediately stored at -80°C until analysis. Blood samples for IL-10 were drawn from radial sheath before selective coronary angiography performed. Measurement of total cholesterol (TC), high density lipoprotein-cholesterol (HDL-C), low density lipoprotein-cholesterol (LDL-C), and triglycerides (TG) was performed as described previously [16, 20, 21]. The serum IL-10 levels were analyzed using a standard enzyme-linked immunosorbent assay (ELISA) using a commercially available kit (R&D Systems, Minneapolis, Minnesota, USA) according to manufacturer's instructions.

Genetic analysis

Genomic DNA was extracted from peripheral blood leukocytes by the salting-out method with minimal modifications. Determination of IL-10 gene -592A/C genotypes was performed by polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP) as

Table 1. Clinical characteristics of the study subjects

Characteristics	SCF (*n* = 41)	NCF (*n* = 209)	*P* value
Age (years)	62.91 ± 10.25	59.76 ± 10.02	0.0679
Male/Female	25/16	130/79	0.8825
BMI (Kg/m^2)	23.11 ± 2.14	22.85 ± 2.28	0.5009
LVEF (%)	61.32 ± 6.24	62.19 ± 6.55	0.4341
Hypertension, n (%)	16 (39.02)	86 (41.15)	0.8003
Diabetes, n (%)	4 (9.76)	7 (3.35)	0.0674
Current smoker, n (%)	12 (29.27)	37 (17.70)	0.0881
TC (mmol/L)	5.05 ± 0.72	4.86 ± 0.60	0.0744
LDL-C (mmol/L)	2.62 ± 0.88	2.51 ± 0.62	0.3365
HDL-C (mmol/L)	1.40 ± 0.28	1.46 ± 0.32	0.2642
TG (mmol/L)	1.77 ± 0.55	1.96 ± 0.68	0.0935
Aspirin, n (%)	36 (87.80)	171 (81.82)	0.3530
Statin, n (%)	8 (19.51)	30 (14.35)	0.4003
β-blocker, n (%)	6 (14.63)	32 (15.31)	0.9121
ACEI/ARB, n (%)	6 (14.63)	26 (12.44)	0.7006
CCB, n (%)	10 (24.39)	37 (17.70)	0.3163
Diuretic, n (%)	4 (9.72)	28 (13.40)	0.5234
IL-10 (ng/L)	1.89 ± 0.18	2.01 ± 0.22	0.0012

BMI, body mass index; LVEF, left ventricular ejection fraction; TC, total cholesterol; LDL-C, low density lipoprotein-cholesterol; HDL-C, high density lipoprotein-cholesterol; TG, triglycerides; ACEI, angiotensin converting enzyme inhibitors; ARB, angiotensin receptor blockers; CCB, calcium channel antagonists; IL-10, interleukin-10.

Table 2. TIMI frame count of SCF and NCF subjects

TIMI frame count	SCF (*n* = 41)	NCF (*n* = 209)	*P* value
LAD (Corrected)	35.24 ± 10.14	21.53 ± 3.41	0.0000
LCX	27.85 ± 9.05	19.92 ± 3.54	0.0000
RCA	26.71 ± 7.26	18.85 ± 3.27	0.0000
Mean TFC	31.83 ± 9.87	20.29 ± 3.55	0.0000

LAD, left anterior descending artery; LCX, left circumflex artery; RCA, right coronary artery; TFC, TIMI frame count.

described previously [10]. 10% of the PCR samples from SCF and NCF groups were sent to direct DNA sequencing.

Statistical analysis

All continuous variables are expressed as the mean and standard deviation (SD). Student's t-test was used to compare continuous variables from two groups. Genotypes and allele frequencies were obtained by direct count. Differences in the distribution of alleles and genotypes between the groups, and deviations from the Hardy-Weinberg equilibrium were assessed by χ^2 test. All significant tests were two-tailed and were considered statistically significant at $P < 0.05$. SPSS for Windows version 11.0 (SPSS Inc., Chicago, IL, USA) was used for all statistical analyses.

Results

Characteristics of the study subjects

The clinical characteristics of all participants enrolled in the study are shown in **Table 1**. No significant differences were seen between the two groups with regard to gender, body mass index (BMI), left ventricular ejection fraction (LVEF), HDL-C, LDL-C, TG, presence of hypertension, smoking status, or medications. Age, presence of diabetes and TC despite a higher trend in the SCF patients did not differ substantially in the two groups. However, compared to the NCF subjects, SCF patients had lower serum IL-10 levels (p = 0.0012).

TIMI frame count of the study population

Patients with SCF had higher TFC in each of the major coronary arteries and mean values compared to the NCF subjects as expected (**Table 2**).

Distributions of IL-10 -592A/C genotypes and allele frequencies

The sequencing results from 10% of the PCR samples in two groups were consistent with the results by PCR-RFLP, as shown in **Figure 1**.

Table 3 summarizes the distributions of IL-10 -592A/C genotypes and allele frequencies for two groups. The genotype distribution among the subjects was in Hardy-Weinberg equilibrium in both the NCF group ($\chi^2 = 0.1540$, $P = 0.6948$) and the SCF group ($\chi^2 = 3.3508$, $P = 0.0672$). The distribution of the IL-10 -592A/C genotypes (AA, AC, and CC) was 46.34%, 41.46%, and 12.20% in the NCF group, and 66.51%, 28.71%, and 4.78% in SCF subjects, respectively ($P = 0.0280$). The frequency of the A allele in the SCF group was significantly higher than that in the NCF group (80.86% vs. 67.07%, $P = 0.0054$). Compared with the CC genotype, the AA genotype had a 3.6579-fold increased risk of SCF (crude odds ratio [OR] = 3.6579, 95% confidence interval [CI] = 1.12881-1.8533, $P = 0.0386$). After being adjusted for age, gender, body mass index, left ventricular

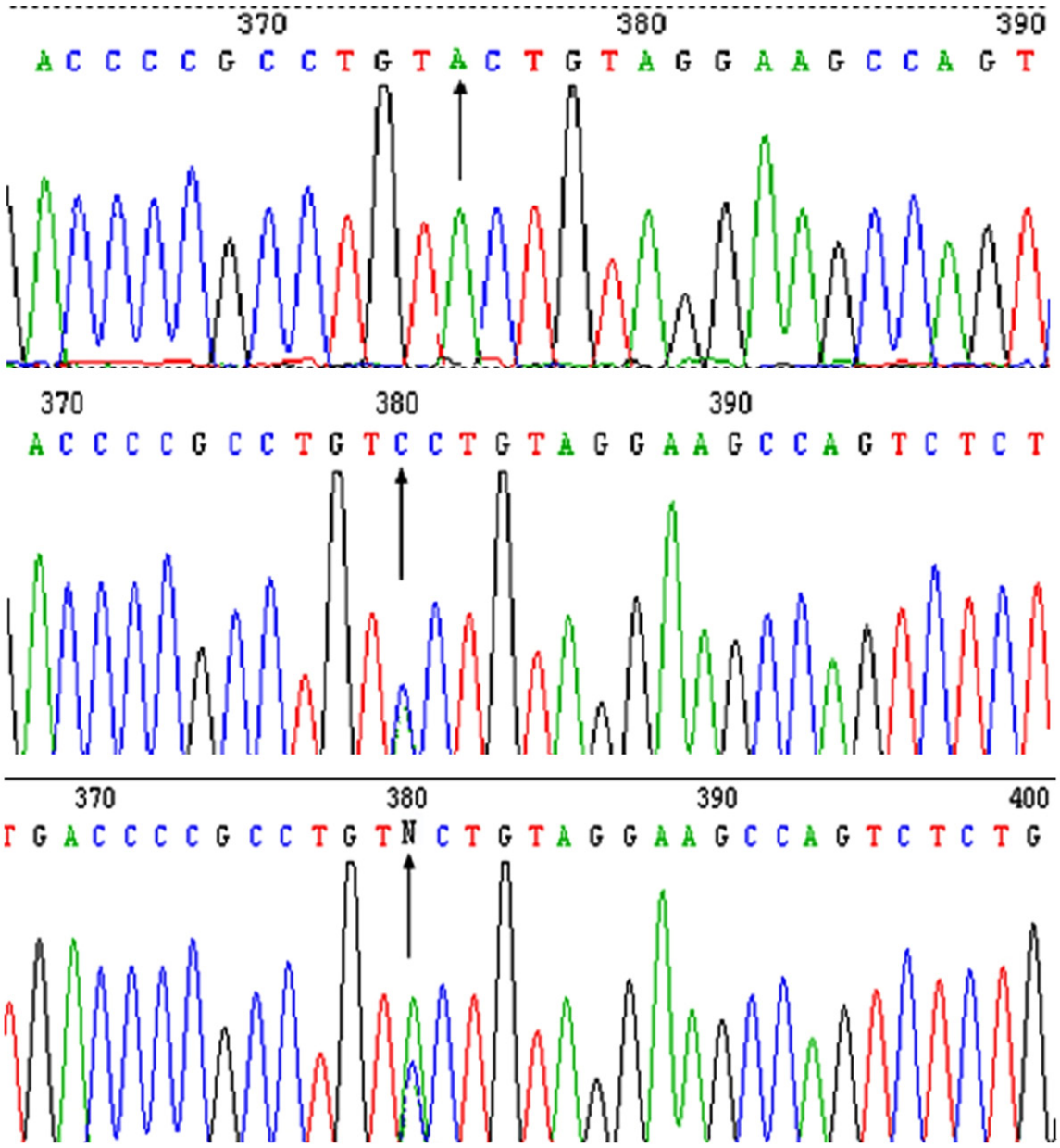

Figure 1. Sequencing results of IL-10 -592A/C genotypes. The sequencing results of the -592A/C genotypes showed that there was only a single A peak in AA genotype, a single C peak in CC genotype and the overlaping A and C peaks in AC genotypes (black arrow).

ejection fraction, prevalence of hypertension and diabetes, serum levels of lipids and IL-10, the association persisted (adjusted OR = 2.9967, 95% CI = 1.0929-8.2173, P = 0.0357) (**Table 4**).

Effects of the different genotypes on serum IL-10 levels

The effects of the different genotypes on serum IL-10 levels in SCF group are depicted in **Figure 2**. Since the numbers of individuals with the CC genotype were small, the carriers of the C allele (AC + CC) were pooled into one group. The average serum IL-10 levels (ng/L) in AA genotype (1.78 ± 0.17) were statistically lower than in AC + CC genotype (2.10 ± 0.20) (P = 0.0000).

Discussion

The major finding of the present study is that there is a strong association between the IL-10 -592A/C polymorphism and risk of SCF. Compared with the CC genotype, the AA genotype

Table 3. Distribution of the IL-10 -592A/C genotypes and alleles in NCF and SCF subjects

Groups	n	Genotypes frequencies (n, %)			Alleles frequencies (n, %)	
		AA	AC	CC	A	C
NCF	41	19 (46.34)	17 (41.46)	5 (12.20)	55 (67.07)	27 (39.93)
SCF	209	139 (66.51)	60 (28.71)	10 (4.78)	338 (80.86)	80 (19.14)
P value			0.0280		0.0054	

had a 3.6579-fold increased risk of SCF. SCF patients with AA genotype also had lower serum IL-10 levels than those with AC + CC genotype. These finding support the hypothesis that low-grade inflammation plays a role in the underlying mechanisms of SCF.

Despite the pathophysiological mechanism of SCF is not consistently determined, several potential hypotheses have been suggested, such as an earlier form of atherosclerosis, the organic or functional dysfunction of small coronary arteries, platelet aggregability, and an imbalance between vasoconstricting and vasodilating factors [22, 23]. Inflammation has been reported to be a major contributing factor in many cardiovascular events and is associated with different clinical settings of CAD, which may be involved in SCF development. In the past few years, a number of studies have tried to shed some light on the possible association between the inflammatory factors and the risk of SCF. In a small pilot study including 17 SCF patients and 20 NCF subjects, serum intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and E-selectin levels were found higher in SCF patients than in NCF subjects, and increased serum levels of ICAM-1, VCAM-1, and E-selectin were significantly correlated with average TFC in SCF patients [24]. In addition, high sensitive C-reactive protein (hs-CRP) and Interleukin-6 (IL-6) were increased and positive correlated with TFC in SCF patients compared with NCF subject [25, 26]. Furthermore, findings that elevated levels of leukocytes [27], neutrophil to lymphocyte ratio (NLR) [28], neutrophil gelatinase-associated lipocalin (NGAL) [14], resistin [29], YKL-40 [3], and soluble CD40 [30] were present in patients with SCF strengthen the argument that inflammation plays a significant role in the development of SCF.

IL-10 is a multifunctional anti-inflammatory cytokine that downregulates cell-mediated immune responses and cytotoxic inflammatory responses [6, 31]. Its effects are mainly directed against functions of mononuclear cells, T lymphocytes and polymorphonuclear leukocytes. Moreover, IL-10 plays a role in inhibition of cell adhesion molecules, tissue factor, fibrinogen, monocyte chemotactic protein-1, matrix metalloproteinase-9, inducible nitric oxide synthase, T-lymphocyte granulocyte-macrophage colony-stimulation factor, and smooth muscle cell proliferation [32, 33]. Furthermore, a potent ability of IL-10 to suppress TNF-α, IL-1α, IL-1β, IL-6, IL-12, and interferon-γ production makes it one of the most important immunoregulator as well as a mediator of inflammatory process [6, 34].

It has been reported that 50-75% of the variation in IL-10 production is genetically controlled [35, 36]. Among the most studied three SNPs in the IL-10 promotor region, -819C/T is in tight linkage disequilibrium with -592A/C [9, 37]. However, the distribution of various genotypes for the IL-10 promoter polymorphic sites differs significantly between ethnic populations [31]. IL-10 -1082 G allele is extremely rare in Han Chinese [11], which is similar to other reports in eastern Asian populations apart from a slight variation [38, 39]. Of 98 Japanese subjects, only 1 GG homozygous was detected, while in Korean population, the frequency of GG genotype was only 0.81%. In contrast, the genotypes distributions of IL-10 -1082 A/G in several Caucasian groups were similar, although the A allele frequency in European Caucasians increased with higher latitudes, with the highest found in a Finnish population [40-42]. The prevalence of IL-10 -592A/C also varies from population to population. In our study of Chinese healthy subjects, the IL-10 -592 A allele frequency was 72.50%, which is very similar to this frequency in southern Chinese (67.00%) [43], Korean (62.00%) [44] and Japanese (67.20%) [45], but markedly different from Caucasians (21.00%) [46]. The ethnic differences suggest that IL-10 promotor polymorphisms could be a useful anthropologic genetic marker. The frequency of the IL-10 -592 A allele was significantly increased in our SCF patients, suggesting that it might represent a candidate genetic marker to predict the risk of SCF.

The present study also found lower serum IL-10 levels in SCF patients than in NCF subjects, and SCF patients with AA genotype had lower serum

Table 4. Relative risk of SCF according to IL-10 -592A/C genotypes

Genotypes	OR (95% CI)	*P* value	OR[a] (95% CI)	*P* value
CC	1.00		1.00	
AC	1.7647 (0.5310-5.8651)	0.5083	1.4216 (0.4390-4.6033)	0.7537
AA	3.6579 (1.1288-11.8533)	0.0386	2.9967 (1.0929-8.2173)	0.0357
AA + AC	2.7639 (0.8922 -8.5621)	0.0784	2.8143 (0.9909-7.9931)	0.0541

[a]Adjusted for age, gender, body mass index, left ventricular ejection fraction, prevalence of hypertension and diabetes, serum levels of lipids and IL-10. OR, odds ratio; CI, confidence interval.

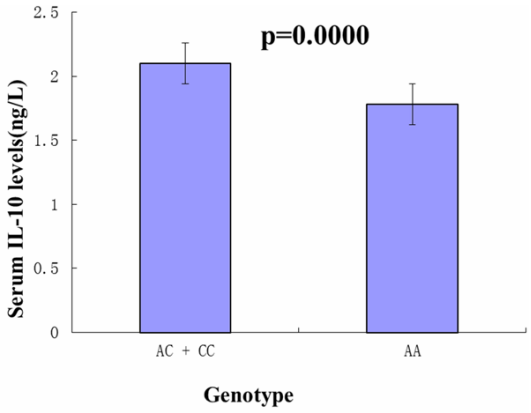

Figure 2. Effects of the different genotypes on serum IL-10 levels in SCF group.

IL-10 levels than those with AC + CC genotype. The position -592 is in an area containing putative binding sites for IL-6 and STAT-1 [47], the two important signaling pathway molecules initiating inflammatory process. Thus, the higher AA genotype frequency and lower IL-10 levels in SCF patients may facilitate the pro-inflammatory cytokines initiating and maintaining SCF phenomenon.

Several limitations need to be addressed. Firstly, the cross-sectional study with no prospective data limits our ability to extract conclusions about the temporal relationships of IL-10 polymorphism and SCF. The relatively limited cohort size restricts the generalizability of our results. Secondly, except IL-10, other inflammatory cytokines might be measured to clarify possible causative mediators. Finally, although all the study subjects were Chinese Han population and thus the possibility of ethnicity as a confounding factor could be excluded, the association of the IL-10 -592A/C polymorphism and SCF in other populations remains unknown and needs further study.

In conclusion, our data support that IL-10 -592A/C polymorphism is associated with SCF and the A allele has increased risk for SCF in Han Chinese. Given the inherent limitations of case-control studies and the complex nature of genetic susceptibility for multi-factors diseases, the prospective and interventional clinical studies with larger sample size are required to be conducted in individual ethnic groups to confirm our observations.

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Disclosure of conflict of interest

None.

Address correspondence to: Dr. Min Pan, Department of Cardiology, Affiliated Hospital of Nantong University, 20 Xisi Road, Nantong 226001, P. R. China. E-mail: panminmd@163.com

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