Correlation between SCAP Genetic Polymorphism and Coronary Artery Disease in a Han population in Xinjiang, China

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Research Article

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Abstract

SREBP cleavage-activating protein (SCAP) plays a vital role in the modulation of cholesterol homeostasis, and cholesterol dysregulation is tightly associated with coronary artery disease (CAD). To investigate the correlation of the genetic polymorphism of *SCAP* with CAD, we conducted a case-control study of 528 CAD patients (case group) and 483 age- and sex- matched subjects from whom CAD was excluded (control group). Three tagSNPs (rs147215799, rs17079634 and rs59586735) in *SCAP* gene were genotyped in all participants, the genotype and allele frequencies of which were compared between two groups to determine their associations with CAD. We found rs17079634 showed significant difference in genotype distribution between the case and control group (*P*=0.016). The difference was most prominent in a dominant model (TT vs. CT + CC, *P*=0.004). After adjustment for confounding factors, the difference remained statistically significant (OR =1.363, 95% confidence interval [CI]:1.022~1.818, *P*=0.035). Whereas no significant associations of the other two SNPs with CAD were observed (*P*=0.393 for rs147215799 and 0.303 for rs59586735, respectively). We drew conclusion that the *SCAP* genetic polymorphism rs17079634 was associated with CAD.

Introduction

Coronary artery disease (CAD) is a leading cause of morbidity and mortality worldwide, and its incidence is still rapidly increasing, especially in China, meanwhile the burden arising from it in terms of mortality and financial cost is increasingly huge[1, 2]. The etiology of CAD is extremely complex and there are still abundant problems awaiting resolution. Overall, numerous studies have indicated that CAD is a complicated polygenic disease and a result of interaction between an individual's genetic composition and a variety of environmental risk factors[3, 4]. Some traditional risk factors for CAD such as hypercholesterolemia and so on have been well-established. Besides these, many genetic alterations have been demonstrated to be associated with CAD and may contribute to CAD susceptibility[5, 6]. However, most of the heritability of CAD remain unexplained, indicating that additional susceptibility loci await identification[7, 8].

As an important macromolecule involved in the development and progression of atherosclerosis which acts as major pathophysiologic events, cholesterol level in vivo is modulated by genetic and environmental factors[9], among them the sterol regulatory element binding protein (SREBP)- SREBP cleavage-activating protein (SCAP) pathway plays a crucial role. Being a major component in this pathway, SCAP binds with SREBPs through its carboxyl-terminal domain to forge SCAP-SREBP complex[10]. Working with insulin induced gene proteins (INSIGs), the complex transfers SREBPs from the endoplasmic reticulum to the Golgi apparatus via feedback regulation of cholesterol levels, finally affecting the synthesis of lipid[11–13].

Given the central role of the SREBP-SCAP pathway in the regulation of cholesterol, the variations in the *SCAP* locus might affect the development and progression of atherosclerosis, thereby contributing to the susceptibility of CAD. Thus far, several studies has investigated the association of genetic variants in

SCAP gene with coronary heart disease[14–16]. However, the SNPs involved in these studies were mainly limited to rs12487736 and the results were at odds with each other. To further systematically evaluate the association of genetic polymorphism in *SCAP* with CAD, here we chose three tagSNPs in *SCAP* and conducted a case-control study in a Han population in Xinjiang, China.

Materials And Methods

Ethical approval of the study protocol

The study was approved by the Ethics Committee of the of the First Affiliated Hospital of Xinjiang Medical University (Xinjiang, China) and was conducted according to the standards of the Declaration of Helsinki. All of the participants provided written informed consents for this study.

Subjects

This study was designed in a case - control study. In total, 1011 subjects were recruited in this study. Of them, 528 patients served as case group who was diagnosed with CAD at the First Affiliated Hospital of Xinjiang Medical University from January 2018 to December 2019, and 483 sex-and age-matched CAD-free individuals served as control group. CAD diagnosis was established by the presence of clinical symptoms such as chest pain and at least one significant coronary artery stenosis of ≥ 50% luminal diameter on coronary angiography,. All of subjects in control group underwent coronary angiography, with no coronary artery stenosis found in them. Patients with congenital or rheumatic heart disease, malignant tumor, multiple organ failure syndrome or other severe illness limiting life expectancy and drug addicts were excluded from this study.

Most patients in the case group had received standardized treatment for coronary heart disease, including lipid-lowering drugs. All of subjects in the control group had not taken lipid-lowering drugs.

The following information was collected: age, gender, smoking, diabetes mellitus and hypertension history, total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), apolipoprotein A□ (ApoA□), apolipoprotein B (ApoB), lipoprotein (a) (Lp(a)) and fasting plasma glucose (FPG).

Biochemical analysis

Serum concentrations of TC, TG, HDL-C, LDL-C, apoA□, apoB, Lp(a), FPG were measured using standard methods in the Department of Clinical Laboratory of the First Affiliated Hospital, Xinjiang Medical University (Xinjiang, China). Hypertension was defined as SBP ≧ 140mmHg and /or DBP ≧ 90mmHg. Smoking was defined as currently smoking cigarettes. Diabetes mellitus was defined as: classic symptoms of hyperglycemia or hyperglycemic crisis plus elevated plasma glucose, includes FPG ≧ 7.0mmol/L, or 2h PG ≧ 11.1mmol/L during OGTT, or a random plasma glucose ≧ 11.1mmol/L; In the

absence of unequivocal symptoms of hyperglycemia, diagnosis requires repeating the glucose measurement on another day.

SNP selection and Genotyping

Blood samples were taken by using anticoagulant ethylene diamine tetraacetic acid (EDTA) tube, and standardized phenol-chloroform method was used to extract genomic DNA from peripheral leukocytes. Three tagSNPs in SCAP, rs147215799, rs59586735, and rs17079634, were selected using Haploview 4.2 software and 1000 Genomes Project (https://www.ncbi.nlm.nih.gov/variation/tools/1000genomes/) with minor allele frequency (MAF) ≥ 0.05 and linkage disequilibrium patterns with r2 ≥ 0.8 as a cutoff[17].

SNP genotyping was performed using an improved multiplex ligase detection reaction method (iMLDR, Genesky Bio-Tech Cod., Ltd., Shanghai, China). We genotyped the selected SNP loci in one ligation reaction. Two multiplex PCR reactions were designed to amplify fragments covering all SNP loci. The PCR programme for both reactions was 95°C, 2 min; 11 cycles × (94°C, 20 s; 65°C– 0.5°C/cycle, 40 s; 72°C, 1 min 30 s); 24 cycles × (94°C, 20 s; 59°C, 30 s; 72°C, 1 min 30 s); 72°C, 2 min; hold at 4°C. The ligation cycling programme was 95°C, 2 min; 38 cycles × (94°C, 1 min; 56°C, 4 min); hold at 4°C. Half a microlitre of ligation product was loaded into the ABI 3730XL and the raw data were analyzed by GeneMapper 4.1. All primers, probes and labelling oligos were designed by and ordered from Genesky Biotechnologies Inc[18].

Statistical analyses

Statistical analyses were carried out using SPSS version 22.0 (SPSS, Chicago, IL). The Hardy-Weinberg equilibrium was assessed by Chi-square test[19]. Continuous variables are expressed as means ± SD in case of normal distribution and as the median (interquartile range) in case of non-normal distribution. Differences in the categorical variables, such as the frequencies of smoking, hypertension, diabetes, and genotypes were analyzed using the chi-square test. After adjusting confounding variables, general linear model analysis was undertaken to test the association between SCAP genotypes and CAD. In addition, a two-tailed *P*-value less than 0.05 was considered to be statistically significant.

Results

Characteristics of study participants

Table 1 showed the clinical characteristics of CAD patients (n = 528) and control subjects(n = 483). Between the two groups, there existed significant differences in following variables: hypertension, diabetes, cigarette smoking, as well as the serum concentrations of TC, HDL-C, LDL-C, ApoA and FPG (all $P < 0.05$). No significant differences were present in following variables between the two groups: ages, sex, serum concentrations of TG, ApoB and Lp(a) (all $P > 0.05$). It was worthwhile noting that serum LDL-C and TC level were significantly higher in control group than in CAD group, the reason for which was the result from the prevalent use of lipid-lowering drugs in CAD patients

Table 1
Clinical and metabolic characteristics of subjects. Statistically significant values are in italics. Data are presented as number of subjects (%) or mean standard ± deviation.

Risk factors	Case	Control	χ^2 or t	P value
Age (years)	56.84 ± 8.58	56.06 ± 1.15	1.234	*0.217*
Male, n (%)	287(54.4%)	263(54.5%)	0.001	*0.974*
Smoking, n (%)	227(43.0%)	176(36.4%)	4.151	*0.042*
Hypertension, n(%)	304(57.6%)	225 (46.6%)	11.178	*0.001*
Diabetes, n(%)	143 (27.1%)	69 (14.3%)	23.131	*< 0.001*
TG (mmol/L)	1.95 ± 1.20	1.91 ± 1.03	0.390	0.697
TC (mmol/L)	4.04 ± 1.04	4.30 ± 1.07	-3.980	*< 0.001*
HDL-C (mmol/L)	1.07 ± 0.29	1.13 ± 0.32	-2.862	*0.004*
LDL-C (mmol/L)	2.50 ± 0.87	2.64 ± 0.83	-2.488	*0.013*
ApoA (mmol/L)	1.20 ± 0.25	1.25 ± 0.25	-3.193	*0.001*
ApoB (mmol/L)	0.83 ± 0.26	0.86 ± 0.28	-1.568	0.117
Lp(a) (mmol/L)	210.33 ± 187.68	192.23 ± 159.55	1.605	0.109
FPG (mmol/L)	6.16 ± 2.59	5.44 ± 1.67	5.168	*< 0.001*

SCAPgenotypes and alleles distributions between CAD group and control subjects

The genotypes and allelse distributions of three selected SNPs in *SCAP* gene were listed in Table 2. The distributions of *SCAP* genotypes for CAD group and control group were all in accordance with predicted Hardy-Weinberg equilibrium (H-WE) values. Of the three SNPs, rs17079634 was shown to be significantly associated with CAD, for its three genotypes, T/T, C/T and C/C, distributed significantly differently between CAD and control groups ($P = 0.016$)(shown in Table 2). If analyzed by specific genetic models (indicated in Table 3), the differences were most prominent in dominant model (TT vs CC + CT, $P = 0.008$), followed by additional model (CT vs TT + CC, $P = 0.02$), whereas not present in recessive model (CC vs CT + TT, $P = 0.27$). Meanwhile, there also existed significant difference in the distributions of the two alleles (T and C) between the two groups ($P = 0.008$). However, genotype distributions of the remaining two

SNPs didn't show different between the two groups (P = 0.393 for rs147215799 and 0.303 for rs59586735, respectively) (shown in Table 2).

Table 2
Distributions of genotypes and alleles of SNPs in subjects. Statistically significant values are in italics.

SNP	rs17079634			rs147215799			rs59586735		
Genotype	T/T	C/T	C/C	T/T	C/T	C/C	T/T	C/T	C/C
CAD	356	157	15	461	66	1	148	274	106
Control	365	109	9	432	51	0	116	271	96
$\chi 2$	8.287			1.866			2.386		
P value	*0.016*			0.393			0.303		

Table 3
Distributions of rs17079634 genotypes according to different genetic model and alleles in two groups. Statistically significant values are in italics.

	Dominant model		Recessive model		Additional model		Alleles	
	TT	CT + CC	CC	CT + TT	CT	CC + TT	T	C
CAD	356	172	15	513	157	371	869	187
Control	365	118	9	474	109	374	839	127
$\chi 2$	8.116		1.041		6.688		8.002	
P value	*0.004*		0.308		*0.010*		*0.005*	

The association ofSCAPrs17079634 with serum lipid profile in control subjects

To explore whether this polymorphism rs17079634 affects serum lipid profile, thereby leading to its association with CAD, we compared the serum lipid profile among different genotypes of this SNP only in the control subjects given CAD patients had prevalently taken lipid-lowering medications such as statins. The three genotypes didn't show significant differences in serum lipid levels (P = 0.934 for TG, 0.910 for TC, 0.284 for HDL-C, 0.992 for LDL-C, 0.805 for ApoA□, 0.468 for apoB and 0.353 for Lp(a), respectively) (data not shown), which suggested that other underlying mechanisms contributed to the association.

The association ofSCAPrs17079634 with CAD after adjustment for confounding factors

To further investigate whether the association of rs17079634 with CAD resulted from the confounding factors, we performed multivariable logistic regression analysis, with the results showed in Table 4. After adjustment for confounding factors such as hypertension, cigarette smoking as well as the serum concentrations of glucose and apoA, the association remained significant in a dominant model (OR = 1.363, 95%CI:1.022 ~ 1.818, *P* = 0.035).

Table 4
The association of *SCAP* rs17079634 with CAD after adjustment for confounding factors. Statistically significant values are in italics.

Factors	B	S.E.	Wald	*P* Value	OR	95% CI
Smoking	.250	.136	3.374	.066	1.284	0.983 ~ 1.677
HP	.310	.147	6.328	*.012*	1.400	1.077 ~ 1.820
DM	.704	.170	17.053	*.000*	2.022	1.448 ~ 2.824
Dominant Model	.310	.147	4.442	*.035*	1.363	1.022 ~ 1.818
Constant	− .699	.216	10.435	*.001*	.497	

Discussion

In this observational, candidate gene association study among 1011 participants, we examined the association between genetic variants in the SCAP locus and CAD susceptibility, and found rs17079634 in SCAP gene was significantly correlated with CAD, specifically, the frequencies of the CT genotype and C allele of *SCAP* rs17079634 were significantly higher in CAD patients than in control subjects, and still significant after the confounding factors were adjusted. This indicated that carriers of *SCAP* rs17079634 CT genotype or C allele have significantly increased risk of CAD.

Abundant studies have demonstrated that, as a complicated polygenic disease, CAD is a consequence of interaction between an individual's genetic composition and a variety of environmental risk factors. As to the former, the foundation for putative causative genes that may be involved in CAD is based on a candidate gene approach. Among the numerous impacts disposing CAD, lipid metabolism dysregulation is believed to play a crucial role, including abnomality in serum lipid profile and excessive cholesterol accumulation in coronary artery contributing to atherosclerosis, etc. Hence, genes involved in cholesterol metabolism are reasonable candidates for CAD. Actually, much attention has been focused on the association of genetic polymorphism in related genes with CAD. Serving as SREBP chaperone, SCAP is the sterol sensing receptor directly interacting with and controlling SREBP transcription factor activation, thereby playing a pivotal role in the modulation of cholesterol and other lipids synthesis. Accordingly,

abnormality in *SCAP* gene and its expression may lead to disturbed cellular cholesterol homeostasis. [20–22]

There has been some studies investigating the correlation of *SCAP* genetic polymorphism with CAD, although the polymorphism involved in these studies is mainly rs12487736 variant and the results were inconsistent. Fan et al investigated the expression of *SCAP* in human atheroma and the association of its allelic variants with sudden cardiac death(SCD), concluded that *SCAP* rs12487736 may contribute to SCD in early middle-aged men[16], but Chen et al didn't found rs12487736 was associated with premature coronary artery disease in a Chinese population[15]. In fact, as of 2020, neither the largest common variant association studies, nor the largest exome-sequencing-based rare, coding variant association studies has nominated SCAP as a genome-wide significant risk locus for CAD [23–24]. In our study, We drew positive conclusion, that is, rs17079634 in SCAP gene was significantly associated with CAD. We speculated that these existing studies were primarily based on European ancestry populations, and future studies in East Asian populations may find relevant in them.

Given the crucial role of SCAP in homeostasis modulation of cholesterol and other lipids, it's natural for researchers to consider if serum lipid levels mediate the correlation between *SCAP* rs17079634 and CAD, in other words, rs17079634 was associated with serum lipid, thereby leading to its correlation with CAD. To make out this, we performed association analysis between rs17079634 and serum lipid profile only in control subjects (for in case group, lipid-lowering agents such as statins have been used prevalently and their serum lipid levels have been affected). We came to a negative conclusion, that is, *SCAP* rs17079634 was not associated with blood lipid levels. Another explanation is, the genetic alterations in *SCAP* may involve in pathogenesis of atherosclerosis through disturbed cholesterol metabolism and accumulation locally at coronary arteries, thereby participating in the development and progression of CAD. Further functional analysis is warranted to verify the hypothesis.

Taken together, we found *SCAP* rs17079634 was strongly associated with CAD, and the carriers of CT genotype or C allele may be at greater risk for CAD. However, several limitations of this study should be mentioned. First and foremost, our study enrolled solely Chinese Han subjects and can not draw ubiquitous conclusion. Second, sample size of the present study was relatively small, which may influence statistical significance and power. Third, the conclusions drew in this work was based only on observational study. Overall, it is reasonable to conduct further association studies with larger sample, rational design and involvement of diverse ethnics with different genetic backgrounds to validate our results, moreover, functional tests should also be put on the agenda to elucidate its underlying molecular mechanisms.

Abbreviations

SCAP	SREBP cleavage-activating protein
SREBP	Sterol regulatory element binding protein
CAD	Coronary artery disease
TG	Triglyceride
TC	Total cholesterol
LDL-C	Low-density lipoprotein cholesterol
HDL-C	High-density lipoprotein cholesterol
ApoA□	Apolipoprotein A□
ApoB	Apolipoprotein B
Lp(a)	Lipoprotein (a)
FPG	Fasting plasma glucose

Declarations

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Authors’contributions

X.D.H., Z.Y.F. and Y.T.M. conceived and designed the study. X.D.H., D.A., Y.H.W. and Y.T.W. performed the study. A.A., B.D.C. and F.L. analyzed the data. X.D.H. wrote the paper. All authors read and approved the final manuscript.

Conflicts of interest

The author(s) declare no competing interests.

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