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Relationship between the acylation-stimulating protein gene and coronary heart disease in the Xinjiang Uygur and Han populations of China

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ABSTRACT. The association of the single nucleotide polymorphism 301T>C in the coding region of the acylation-stimulating protein (ASP) gene with coronary heart disease (CHD) was investigated in the Uygur (385 CHD patients and 483 control subjects) and Han (390 CHD patients and 439 control subjects) populations of China. The frequency of the CC and CT genotypes was significantly higher in patients with CHD compared to the control group (55.3 *vs* 46.2%, P = 0.001) in the Uygur population, but in the Han population, the frequency was significantly higher in the control group (51.7 *vs* 24.4%, P < 0.001). In addition, the C allele was significantly associated with CHD in the Uygur population (C allele: 33.8 *vs* 26.2%, T allele: 66.2 *vs* 73.8%; P = 0.004) and in the Han population (C allele: 14.5 *vs* 30.3%, T allele: 85.5 *vs* 69.7%; P < 0.001). The CC genotype was independently associated with increased risk of coronary artery disease when adjusted for other cardiovascular risk factors [odds ratio (OR) = 2.189, 95% confidence interval (CI) = 1.251-3.830, P = 0.001] in the Uygur population, but was a protective factor

for CHD in the Han population (OR = 0.373, 95%CI = 0.187-0.745, P = 0.005). In conclusion, the 301T>C polymorphism of the ASP gene that influences the serum triglycerides level in the Uygur population, is associated with the development of CHD, and the CC genotype might be a risk factor of CHD.

Key words: Acylation-stimulating protein; Triglyceride; Han; Uygur; Coronary heart disease

INTRODUCTION

Epidemiological studies have indicated that the incidence of coronary atherosclerotic heart disease (CHD) is associated with environmental and genetic factors. CHDs are complex disorders. Hypertriglyceridemia (HTG) is a major independent risk factor for diabetes, hypertension, hyperlipemia, and CHD (Frayn, 2001; Redinger, 2008); atherosclerosis, a prominent feature of dyslipidemia, is commonly observed in CHD, and also an independent risk factor of coronary artery disease (CAD). The acylation-stimulating protein (ASP) gene, located in the C3a gene cluster, is an important candidate gene contributing to HTG. ASP results in elevated plasma triglyceride (TG) levels as a non-competitive inhibitor of lipoprotein lipase (LPL), and is the rate-limiting enzyme in TG-rich lipoprotein (TRL) catabolism (Yasruel et al., 1991). Some studies have demonstrated that ASP is the main anabolic stimulator of TG storage in adipose tissue and is produced by adipocytes (Cianflone et al., 2003). ASP stimulates TG synthesis via the ASP receptor, C5L2, a seven-transmembrane G protein-coupled receptor (Kalant et al., 2003, 2005). Several key signaling proteins have been identified downstream of ASP, including phospholipase C, phosphatidylinositol-3 kinase, Akt, and protein kinase C, resulting in increased glucose transport and diacylglycerol acyltransferase activity (Maslowska et al., 2006). ASP is identical to C3a desArg, and produced through the interaction of the precursor protein C3, Factor B, and adipsin (also known as Factor D), components of the alternative complement immune pathway, which are secreted by the adipose tissue. The aim of the present study was to determine the frequencies of polymorphisms of the ASP gene in the Chinese Uygur and Han populations in order to investigate the associations with variations in plasma lipid and lipoprotein levels in patients with CHD. Accordingly, homozygotes for risk alleles of the single nucleotide polymorphism (SNP) 301T>C of the ASP gene may have an increased risk of developing CHD.

MATERIAL AND METHODS

Subjects

All subjects in the control group (439 Han and 483 Uygur) were unrelated age- and gender-matched individuals who were selected via health screenings at the Outpatient Department of the First Hospital of XinJiang Medical University, Urumqi, China from January 2007 to March 2010; none of the subjects had coronary vascular diseases. The case group consisted of CHD patients (385 Uygur and 390 Han) with significant coronary stenosis determined by cardiovascular angiography (according to stenosis ≥50% in at least one coronary artery) at the Department of Endocrinology of the First Hospital of XinJiang Medical University, Urumqi. None of the patients were related.

The study was approved by the Ethics Committee of the First Hospital Affiliated to Xinjiang Medical University. Neither the patients nor the controls had congenital heart disease, rheumatic heart disease, heart failure, multiple organ failure, or other general illnesses.

Data collection

The serum concentrations of TG, cholesterol (CHOLC), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C), as well as blood urea nitrogen (BUN), creatinine (Cr), and uric acid (UA) levels of all subjects were measured with an Automatic Biochemistry Analyzer (Olympus 5400) and a GmbH diagnostic kit (Roche) in the Central Laboratory of the First Affiliated Hospital of XinJiang Medical University.

Genotyping

Fasting venous blood was collected in 5-mL EDTA tubes, and genomic DNA was isolated with a Pure Gene kit (Gentra Systems Inc.). Sequence information for use as a reference template was obtained from the Ensembl Genome Browser. Sequencing primers were designed using the Primer 5.0 software. The sense primer was 5'-CGCCAGTGAGGCAATGTG-3' and the antisense primer was 5'-CCCAGCGAGGCAGTTCTT-3'. Extraction of genomic DNA from peripheral blood samples was conducted as described previously (Cianflone et al., 2003; Yang et al., 2006). The polymerase chain reaction (PCR) was carried out with 50 ng genomic DNA in a 20 μL reaction containing 10 μL Power Mix (Beijing Biotech; Beijing, China), 9.5 μL distilled water, and 0.2 μL each forward and reverse primer. A Gene Amp 9700 thermal cycler (Applied Biosystems; Foster City, CA, USA) was used for PCR amplification. The program consisted of an initial denaturation step at 95°C for 5 min, 35 cycles at 95°C for 30 s, 56°C for 30 s, and 72°C for 1 min, followed by a final extension step at 72°C for 10 min. A 1615-base pair (bp) product was amplified and purified using ExoSAP-IT (Amersham Biosciences) according to manufacturer instructions before it was used as a template for sequencing. Sequencing reactions were performed by BGI-Beijing (Beijing, China; http://www.genomics.cn).

The PCR products of the 301T>C polymorphism sites were digested with the restriction enzyme *Rsa*I (Fermentas; Beijing, China) at 37°C for 16 h, separated by electrophoresis on 2% agarose gel, and visualized by ethidium bromide staining. Fragments of 320 bp for the TT wild homozygote (absence of *Rsa*I cutting site) and of 212 and 108 bp for the CC mutant homozygote (presence of *Rsa*I cutting site) were produced. The TC heterozygote showed three fragments of 320, 212, and 108 bp (Figure 1).

Statistical analysis

All data were analyzed using the SPSS 17.0 software (SPSS; Chicago, IL, USA). Hardy-Weinberg equilibrium was assessed by the chi-square test. Measurement data are reported as means ± SD, and differences between CHD patients and control subjects were assessed with the independent sample Student's *t*-test. Fasting TG levels were log-transformed using natural logarithms for analyses and are presented as geometric means with the inter-quartile range (25th-75th quartile). Differences in enumeration data between the CHD group and the control group were analyzed using the chi-square test, as the differences in the distributions of genotypes and alleles between the two groups. Logistic regression analyses were used to assess the contribution of the major risk factors.

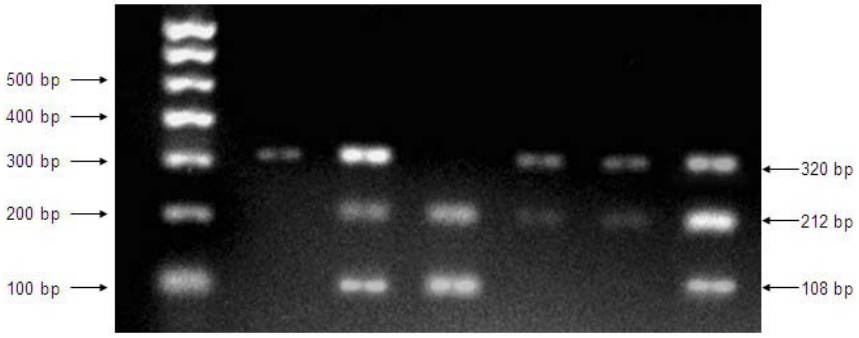

Figure 1. Restriction fragment length polymorphism analysis for genotype determination. CT genotype shows three bands of 320, 212, and 108 bp (2, 4, 5, and 6); CC genotype shows two bands of 212 and 108 bp (3); TT genotype shows one band of 320 bp (1).

RESULTS

Participant characteristics

The general characteristics of the study subjects (Han and Uygur) are presented in Table 1. In Han subjects, there were no significant differences in gender, body mass index (BMI), diastolic blood pressure (DBP), TG, UA, and Cr between the two groups ($P > 0.05$). However, there were significant differences in age, hypertension, diabetes, smoking, drinking, glucose, CHOLC, HDL-C, LDL-C, and BUN between the two groups ($P < 0.05$). In Uygur subjects, there were no significant differences in gender and BUA between the two groups ($P > 0.05$). However, age, hypertension, diabetes, smoking, drinking, glucose, CHOLC, TG, HDL-C, LDL-C, and Cr values in the two groups were significantly different ($P < 0.05$).

Table 1. Characteristics of the participants.

	Han population				Uygur population			
	Control (N = 439)	CHD (N = 390)	χ^2 or t	Pvalue	Control (N = 483)	CHD (N =385)	χ^2 or t	P value
Age, mean (SD)	57.91 (12.17)	60.71 (10.32)	-3.532	0.000	50.42 (9.42)	54.65 (10.07)	-6.367	0.000
Gender, female (%)	107 (24.4)	89 (22.8)	0.599	0.276	90 (18.6)	67 (17.4)	0.219	0.640
Hypertension, N (%)	227 (52.1)	253 (65.0)	14.223	0.000	113 (23.5)	147 (38.2)	21.781	0.000
Diabetes, N (%)	132 (30.3)	198 (51.0)	36.536	0.000	68 (14.9)	89 (23.1)	9.164	0.002
Smoking, N (%)	167 (38.4)	218 (55.9)	25.322	0.000	133 (27.7)	218 (56.6)	74.079	0.000
Drinking, N (%)	93 (21.4)	135 (34.8)	18.257	0.000	72 (15.1)	143 (37.2)	64.795	0.000
BMI, mean (SD)	25.62 (3.30)	184 (37.4)	1.544	0.123	25.60 (3.86)	27.31 (4.30)	-5.299	0.000
SBP, mean (SD)	134.37 (18.59)	141.80 (31.56)	-3.786	0.000	126.22 (16.11)	138.01 (27.59)	-6.279	0.000
DBP, mean (SD)	84.28 (14.44)	85.72 (17.735)	-1.134	0.257	80.66 (13.63)	84.97 (16.78)	-4.302	0.001
Glucose, mean (SD)	4.75 (0.79)	6.29 (2.50)	-12.202	0.000	5.32 (1.74)	5.84 (2.08)	-3.789	0.000
TG, mean (SD)	1.876 (1.48)	1.98 (1.30)	-1.114	0.266	1.72 (1.87)	1.98 (1.11)	-2.358	0.019
TC, mean (SD)	4.581 (1.02)	4.153 (1.03)	5.986	0.000	4.59 (1.33)	4.25 (0.98)	4.006	0.000
HDL-C, mean (SD)	1.394 (0.41)	1.138 (0.33)	9.687	0.000	1.23 (0.41)	1.03 (0.57)	5.54	0.000
LDL-C, mean (SD)	3.121 (0.97)	2.516 (0.85)	9.277	0.000	2.72 (0.89)	2.58 (0.92)	2.11	0.035
UA, mean (SD)	334.86 (95.39)	330.11 (86.60)	0.744	0.457	281.46 (89.87)	328.58 (81.63)	-7.65	0.000
Cr, mean (SD)	74.85 (18.81)	77.98 (26.31)	-1.980	0.048	73.61 (23.68)	77.94 (18.93)	-2.82	0.005
BUN, mean (SD)	4.997 (1.46)	5.330 (1.70)	-3.021	0.003	4.98 (1.62)	5.15 (1.81)	-1.428	0.154

BMI = body mass index; SBP = systolic blood pressure; DBP = diastolic blood pressure; TG = triglyceride; TC = total cholesterol; HDL-C = high-density lipoprotein cholesterol; LDL-C = low-density lipoprotein cholesterol; UA = uric acid; Cr = creatinine; BUN = blood urea nitrogen; CHD = coronary heart disease.

Distribution of genotype and allele frequencies of the polymorphic site in the CHD and control groups

Table 2 shows the genotype and allele distributions of the ASP (301T>C) gene polymorphic site in the study population. The genotype distribution in each group conformed to Hardy-Weinberg equilibrium (P > 0.05 in the CHD group and the control group). In Han subjects, the frequencies of the C/C, C/T, and T/T genotypes of the ASP (301T>C) polymorphism were 4.6, 19.7, and 75.6%, respectively, in the CHD group, and 8.9, 42.8, and 48.3%, respectively, in the control group (P < 0.001). The frequency of the C allele was lower in the CHD group than in control subjects in the Han population (14.5 *vs* 30.3%, P < 0.001), but the frequency of the C allele was higher in CHD patients than in Uygur control subjects (33.8 *vs* 26.2%, P = 0.004). Furthermore, we found that patients with the CC genotype had a higher risk of CHD (CC *vs* TT: OR = 2.189, P = 0.001, 95%CI = 1.251-3.830) in Uygur subjects. However, in the Han population, the CC genotype showed a protective effect in CHD patients (CC *vs* TT: OR = 0.373, P = 0.005, 95%CI = 0.187-0.745) (Table 3). Furthermore, there were significant differences in smoking, hypertension, TG, and HDL-C between the two groups in both the Uygur and Han populations (P < 0.05), as shown in Table 3.

Table 2. Distribution of genotype and allele frequencies of polymorphic site of acylation-stimulating protein gene.

Group		N	Genotype (N, %)				P	Allele (Frequency)		P
			CC	CT	TT	CC+CT		C	T	
Han	Control	439	39 (8.9)	188 (42.8)	212 (48.3)	227 (51.7)	<0.001	266 (30.3)	612 (69.7)	<0.001
	CHD	390	18 (4.6)	77 (19.7)	295 (75.6)	95 (24.4)		113 (14.5)	667 (85.5)	
Uygur	Control	483	29 (6.0)	194 (40.2)	260 (53.8)	223 (46.2)	0.001	252 (26.2)	714 (73.8)	0.004
	CHD	385	47 (12.2)	166 (43.1)	172 (44.7)	213 (55.3)		260 (33.8)	510 (66.2)	

CHD = coronary heart disease.

Table 3. Logistic regression analysis of association between the acylation-stimulating protein genotypes and risk of coronary heart disease.

	Uygur						Han					
	B	S.E.	Wald	P	OR	95%CI	B	S.E.	Wald	P	OR	95%CI
CC	0.784	0.285	7.541	0.001	2.189	1.251-3.830	-0.985	0.352	7.829	0.005	0.373	0.187-0.745
Smoking	1.127	0.164	47.083	0.000	3.086	2.237-4.258	0.751	0.172	19.182	0.000	2.121	1.515-2.967
Hpertention	0.547	0.175	9.523	0.002	1.717	1.218-2.421	0.526	0.175	9.075	0.003	1.693	1.202-2.384
TG	0.135	0.056	5.781	0.016	1.145	1.025-1.279	0.308	0.087	12.555	0.000	0.735	0.620-0.871
HDL	-0.578	0.223	6.160	0.013	0.561	0.355-0.855	-1.515	0.264	32.926	0.000	0.220	0.131-0.369
Constant	0.688	0.394	3.052	0.081	1.991		2.720	0.426	40.697	0.000	15.183	

TG = triglyceride; HDL = high-density lipoprotein.

DISCUSSION

This is the first study to evaluate functional polymorphisms of the ASP gene in relation to CHD susceptibility in the Uygur and Han populations of China. The results demonstrated that the 301T>C polymorphism of the ASP gene is significantly associated with an increased risk of CHD in the Uygur population.

Much attention has been focused on the association of genetic polymorphisms with CHD. ASP is increased in diabetes, cardiovascular disease, hyperthyroidism, and polycystic

ovary syndrome (Yang et al., 2006). Cellular studies have demonstrated that ASP is a main anabolic stimulator of TG storage in adipose tissue and is produced by adipocytes.

ASP stimulates TG synthesis via the ASP receptor, C5L2, a seven-transmembrane G protein-coupled receptor (Cianflone et al., 2003; Kalant et al., 2003). Several key signaling proteins have been identified downstream of ASP, including phospholipase C, phosphatidylinositol-3 kinase, Akt, and protein kinase C (Kalant et al., 2005), culminating in increased glucose transport and diacylglycerol acyltransferase activity (Maslowska et al., 2006). ASP is identical to C3a desArg and is produced through the interaction of the precursor protein C3, Factor B, and adipsin (also known as Factor D), components of the alternative complement immune pathway, which are secreted by adipose tissue.

We identified the 301T>C polymorphism and assessed the association between ASP and CHD in the Han and Uygur populations. The frequency of the CC genotype was significantly higher in CAD patients than in control subjects in the Uygur population. However, in the Han group, the frequency of the CC genotype was lower in CAD patients than in control subjects. This indicated that the risk of CHD was increased in Uygur subjects carrying the C allele. After adjustment for other cardiovascular risk factors, logistic regression analyses suggested that compared to the TT genotype, the CC genotype is associated with a higher risk of CAD (OR = 2.189, P = 0.001, 95%CI = 1.251-3.830) in the Uygur population. By contrast, in the Han population, the CC genotype has a higher protective effect for CAD compared to the TT genotype (OR = 0.373, P = 0.005, 95%CI = 0.187-0.745).

Lipid disorders are associated with high TG and TC levels; therefore, meals with high levels of saturated fatty acids will obviously increase lipid levels. We found that the TG level was higher in CHD patients than in control subjects in the Uygur population ($P < 0.05$). Previous studies have found that the main reasons for differences in individual lipid levels are associated with diet structure, financial conditions, and social environment (Linn et al., 1989; Kesteloot et al., 1989). The diet structure of the Uygur population in this study is mainly comprised of mutton and milk, and animal fat accounts for 32% of the meat intake quantity (He and Zhang, 1989), which may be the principal reason contributing to the high incidence of hyperlipemia in the Xinjiang population of China. Logistic regression analyses suggested that smoking is a high risk factor for CHD in the Han and Uygur populations. Studies have found that smoking has a negative impact on lipids (Handa et al., 1990; Imamura et al., 2000); it can significantly increase HDL-C levels and also the standard level of total cholesterol (Wu et al., 2001).

The results of our study demonstrated that the levels of plasma TG were much higher in patients with the CC genotype in the Uygur population. As previously demonstrated (Cianflone et al., 1989; Sniderman and Cianflone, 1994), the key genes in the conversion of complement C3 to its ASP form (C3a desArg) are all produced by adipocytes: C3, factor B (FB), adipsin (or factor D), and carboxypeptidase N (CPN1). Genetic polymorphisms may vary among different ethnic groups. In our study population, the frequencies of the 301T>C polymorphism for the CC, CT, and TT genotypes were 6.0, 40.2, and 53.8%, respectively in the control subjects, and were 12.2, 43.1, and 44.7%, respectively in the CHD patients. The genotype frequencies for CC+CT genotypes were significantly higher in the CHD group than in the control group (46.2 and 55.3%, respectively, P = 0.001). After adjustment for age, gender, smoking, hypertension, diabetes mellitus, and hypercholesterolemia, the C allele carriers had a higher risk of developing CHD compared to TT homozygotes. It has also been found that ASP is highly expressed in the shoulder regions of advanced atherosclerotic lesions, which suggested that this potent matrix-degrading enzyme also contributes to plaque instability. Similar

to the findings of Fallah et al. (2010), we did not find any significant effect of the 301T>C polymorphism on the number of diseased vessels in the Uygur population.

In conclusion, this study suggests that the ASP 301T>C polymorphism could be associated with susceptibility to CHD in the Uygur and Han populations of China, and that 301C allele carriers might be at high risk of developing CHD in the Uygur population. This result may broaden the knowledge of genetic variants and disease-association studies. Undertaking genome-wide association studies in different populations certainly deserves investigation.

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Conflicts of interest

The authors declare no conflict of interest.

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