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Association between DNA variant sites in the apolipoprotein A5 gene and coronary heart disease in Chinese

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Abstract

The recently discovered apolipoprotein A5 (*APOA5*) gene has been shown to be important in determining plasma triglyceride levels, a major cardiovascular disease risk factor. We searched for possible associations of the *APOA5* gene polymorphisms S19W and −1131T>C with coronary heart disease (CHD) in a Chinese population. A total of 483 Chinese CHD patients and 502 control non-CHD subjects were genotyped by polymerase chain reaction–restriction fragment length polymorphism for these 2 single nucleotide polymorphisms. We found that the minor allele 19W was observed only in CHD patients and not in controls, with allelic frequencies of 0.047 and 0.000, respectively ($P < .000001$), and the minor allele −1131C was significantly higher in CHD patients than in controls (0.391 vs 0.299, $P < .0001$). These results suggest that both the S19W and −1131T>C variations in the *APOA5* gene are associated with the CHD and appear to be 2 genetic risk factors for CHD susceptibility in Chinese. Moreover, we found that triglyceride levels were significantly higher in −1131C carriers than in −1131T subjects of the control group and that high-density–lipoprotein cholesterol was decreased in −1131C carriers among CHD patients.

Keywords: Apolipoprotein A5; Triglycerides; Coronary heart disease; Single nucleotide polymorphisms; Chinese population study

1. Introduction

The apolipoprotein A5 gene (*APOA5*) plays a key role in determining plasma triglyceride (TG) concentrations. In *Apoa5*(−/−) knockout mice, serum TG levels are 4 times higher than those of normal mice, whereas transgenic mice expressing human *APOA5* have TG levels that are only approximately one third of normal [1,2]. Clinical studies have also suggested an important role for *APOA5* in determining plasma TG concentrations. When single nucleotide polymorphisms (SNPs) and their haplotypes across the *APOA5* locus have been studied in human beings, some of SNPs were found to be significantly associated with plasma TG levels in different ethnic populations [3,4]. However, no studies on the possible association of *APOA5* SNPs with coronary heart disease (CHD) in Chinese have been so far reported.

It has become increasingly clear that both genetic and environmental factors contribute to the etiology of CHD. Epidemiological studies have shown that hypertriglyceridemia is a major independent risk factor for CHD [5]. The relationship between elevated plasma TGs and CHD risk has been confirmed by meta-analysis, identifying TG as an independent CHD risk factor [6]. With the discovery of the *APOA5* gene and its association with TG, several common *APOA5* SNPs have been identified [3]. Subsequently, many

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studies in different ethnic groups have demonstrated associations of some of the *APOA5* SNPs with plasma TG and very low-density lipoprotein cholesterol levels [7-13]. Furthermore, 2 haplotypes of the *APOA5* gene, defined by 2 tag SNPs—S19W (GenBank_ID: ss4383597, in the third exon, also reported as 56C>G) and −1131T>C (GenBank_ID: ss3199915, in the promoter, previously reported as SNP3)—are reported to have a significant influence on human plasma TG levels [4].

In the present study, we report an association of *APOA5* SNPs with CHD and the correlation of both the S19>W and −1131T>C mutations with levels of the plasma lipids and lipoproteins in Chinese CHD patients and controls.

2. Materials and methods

2.1. Subjects

Four hundred eighty-three unrelated CHD patients were selected between 2000 and 2004 from the West China Hospital, Sichuan University, for the study by coronary angiography, using the Judkins technique [14]. Individuals having any major coronary artery branch (left anterior descending artery, left circumflex artery, right coronary artery) with at least 1 stenosis of >60% were qualified as CHD patients. In addition, 502 unrelated age- and gender-matched subjects, selected via health screening at the same hospital and free of any clinical or biochemical signs of CHD, were used as controls for the study. None of the patients enrolled in this study was taking hypolipidemic drugs before coronary angiography or measurements of their lipid profiles. The study was approved by the Internal Ethical Review Board of the West China Hospital, Sichuan University, and signed informed-consent forms were obtained from all subjects studied.

2.2. Measurement of lipids and lipoproteins

After an overnight fast, baseline blood samples were collected from all CHD patients and controls. Plasma was separated and used immediately for lipid and lipoprotein analysis. The levels of plasma total cholesterol (TChol), TG, and high- and low-density lipoprotein cholesterol (HDL-C and LDL-C) were measured by an automated chemistry analyzer (Olympus AU5400, Japan) with an enzymatic kit (Roche Diagnostics GmbH).

2.3. DNA preparation, polymerase chain reaction amplification, and genotyping

Genomic DNA was isolated from peripheral blood leukocytes, using the "salting-out" procedure [15], and then stored at 4 °C for use. *APOA5* loci genotyping was performed using the polymerase chain reaction (PCR)–restriction fragment length polymorphism method. The PCR was carried out as described [4]. For amplification of the fragment containing 56C>G (S19W), the primers were as follows: forward, 5′–GGC TCT TCT TTC AGG TGG GTC TCC G-3′; reverse, 5′-GCC TTT CCG TGC CTG GGT GGT-3′. The 157-bp PCR products were digested with 6 U of *Taq*I (MBI Fermentas) for 4 hours at 65 °C. After digestion, the digested products were separated by electrophoresis on a 3% agarose gel stained with ethidium bromide and visualized on an ultraviolet transilluminator. The fragments of 134 and 23 bp represented the Ser-19 allele, and a single uncut product represented the Try-19 allele. The primers for amplification of the fragment containing −1131T>C were as follows: forward, 5′-GGA GCT TGT GAA CGT GTG TAT GAG T-3′; reverse, 5′-CCC CAG GAA CTG GAG CGA AAT T-3′. The PCR products were then directly digested with *Mse*I (New England Biolabs, USA) at 37 °C overnight. When the 154-bp PCR-amplified fragment was digested, it produced fragments of 133 and 21 bp for the −1131T allele, and the single uncut product represented the −1131C allele.

2.4. Statistical analysis

The data of mean and standard deviation are presented as $\bar{\chi} \pm s$ and percentages as %. Because TG levels were not normally distributed, they were logarithmically transformed before the statistical analysis, but untransformed values are presented in the tables. Allele frequencies were determined by counting alleles and calculating sample proportions. Hardy-Weinberg equilibrium was confirmed using the χ^2 test. Differences in lipid and lipoprotein values of the various genotypes were evaluated by 1-way analysis of variance (ANOVA) and the Student-Newman-Keuls SNK test. Differences of genotypic and allelic distribution between patients and controls were analyzed by χ^2 or Fisher exact tests (when appropriate). The odds ratios for CHD were derived from logistic regression analysis. All statistical analyses were carried out with SPSS 11.0 software (SPSS Inc, Chicago, Ill).

3. Results

3.1. General and clinical biochemical characteristics of the patients and controls

The clinical characteristics and plasma lipid levels of the CHD patients and controls are shown in Table 1. As can be

Table 1
General and clinical biochemical characteristics of CHD patients and controls

	CHD patients	Controls	*P*
No. of subjects	483	502	–
Sex (M/F)	285/198	276/226	–
Age (y)	54.2 ± 6.3	54.4 ± 5.8	NS*
BMI (kg/m^2)	25.92 ± 3.24	23.45 ± 2.83	<.01
TG (mmol/L)	1.98 ± 0.88	1.57 ± 0.83	<.01
TChol (mmol/L)	5.51 ± 0.78	5.13 ± 0.80	<.01
HDL-C (mmol/L)	1.13 ± 0.46	1.67 ± 0.83	<.01
LDL-C (mmol/L)	3.21 ± 0.86	3.04 ± 0.92	<.01

M/F indicates male/female; BMI, body mass index.

* NS indicates no significant difference ($P > .05$).