ABCG5/8 variants are associated with susceptibility to coronary heart disease

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Abstract. ATP-binding cassette sub-family G member 5 (ABCG5) and ABCG8 are members of an ATP-binding cassette transporter superfamily. *ABCG5* and *ABCG8* variants affected serum levels of cholesterol and were considered as risk factors for coronary heart disease (CHD). The present control study analyzed *ABCG5* and *ABCG8* variants in a population for association with the risk of CHD. A total of 417 CHD patients and 267 controls were recruited for genotyping of four single nucleotide polymorphisms (SNPs; i.e. i7892T>C in ABCG5 and Tyr54CysA>G, Thr400LysC>A and 5U145A>C in ABCG8) using quantitative PCR high-resolution melting (qPCR-HRM). Serum lipid levels were measured using an automatic biochemical analyzer. The association of *ABCG5/8* variants with lipid levels was analyzed using a Chi-square test. The impact of candidate ABCG5/8 SNPs on CHD was evaluated in a dominant genetic model with stepwise multiple regression analysis. Subgroup analyses were performed with regard to these SNPs, tobacco smoking status, alcohol consumption and gender. Genotypic and allelic frequencies of ABCG8 Thr400LysC>A were significantly different (P<0.05) between CHD patients and controls. CC homozygotes of the ABCG8 Thr400LysC>A SNP had greater triglyceride levels than CA/AA carriers with CHD. Logistic analysis revealed CHD risk was significantly higher in CC homozygotes of ABCG8 Thr400LysC>A than in carriers of the A allele (adjusted P=0.048; OR=2.034; 95% CI=0.983-4.207). Furthermore, there was a significant gene-tobacco smoking interaction. CC homozygotes of ABCG8 Thr400LysC>A SNP had significantly higher triglyceride concentrations (P=0.012) and an increased risk of CHD than tobacco smoking carriers of the A allele. The data from the current study suggested that ABCG8 Thr400LysC>A SNP genetic variants modulated plasma triglyceride levels and thereby affected CHD risk in the population studied. The genetic variant of ABCG8 also contributed to CHD risk through interaction with tobacco smoking.

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Introduction

Coronary heart disease (CHD) is a significant health problem and one of the leading causes of morbidity and mortality in China. The most common risk factors include tobacco smoking, alcohol consumption, family history, hypertension, obesity, diabetes, lack of exercise, stress and hyperlipidemia. These risk factors interact with host genetic factors to impact the development of CHD. To date, >30 common genetic susceptibility loci have been identified by genome-wide association studies (GWAS) and are independently associated with CHD risk (1-4), however, the majority are based on European populations. For the Chinese Han population, polymorphisms in chromosome 9p21 (5,6) are the only loci demonstrated to have a CHD risk, with the carriers having a 30% increased risk of CHD compared with non-carriers (7). Thus, further studies on gene polymorphisms are required in order to identify those that result in susceptibility to CHD by interacting with environmental risk factors and therefore, lead to the development of preventive and therapeutic strategies for CHD.

Towards this end, the present study focused on ATP-binding cassette sub-family G member 5 (ABCG5) and ABCG8, loci that are localized on human chromosome 2p21. ABCG5 and ABCG8 are hemi-transporters that regulate the absorption of sterols from the intestine and promote the canalicular conversion and secretion of cholesterol and plant sterols into bile (8). Their proteins are exclusively expressed in the intestine and liver cells (9) and, together with a number of transporter proteins, are important in the maintenance of lipid homeostasis in the human body. Mutations in either gene is associated with sitosterolemia, accumulation of dietary cholesterol, accelerating atherosclerosis and premature CHD (10,11). Several studies have demonstrated that common variants in ABCG5/8 correlate with serum lipid levels with concordant changes in the risk of CHD. The populations in these studies were predominantly Caucasian (12-16), Chilean (17), Czech (18) or Taiwanese consuming the traditional Chinese Southern diet (19) and the results from diverse ethnic groups were not consistently reproducible. For example, several previous studies in China indicated that polymorphisms of *ABCG8* are associated with

the upregulated absorption and esterification of cholesterol in the small intestine and increase the incidence of gallstone disease in a gender-specific manner (20,21). However, the responsible variants and the molecular mechanisms underlying the incidence of CHD have not yet been identified.

Due to the colder climate, the Han Chinese population in Northern China consume a high-fat, high-energy and low-fiber diet compared with the Southern Han Chinese. Since the launch of China's reform and opening-up policies in 1978, the Han Chinese have adopted a Westernized diet and other habits, including smoking, alcohol consumption, accelerated aging, increased working hours and complex interpersonal relations, increasing the incidence and risk of CHD. Indeed, a number of studies have indicated that dietary constituents perturb cholesterol homeostasis and consequently affect expression of hepatic transporters. Yamazaki *et al* (22) demonstrated that a high calorie diet induces lipid loading in the liver and causes significant increases in the expression of ABCG5/8 in bile canaliculi compared with individuals on an ordinary diet. Another study demonstrated that the increased expression of ABCG5/8 attenuates diet-induced hypercholesterolemia in Ldlr-/- mice, resulting in a significant reduction in plasma levels of cholesterol and aortic atherosclerotic lesions (23). Thus, the present study hypothesized that ABCG5/8 loci polymorphisms are crucial in the maintenance of lipid homeostasis and CHD susceptibility in the northern Han Chinese population. The present study aimed to confirm the novel susceptibility variants for CHD that were previously identified in a European population using a case-control study. The association of four candidate variants in ABCG5/8 genes, including i7892T>C in ABCG5 and Tyr54CysA>G, Thr400LysC>A and 5U145A>C in ABCG8 were evaluated using quantitative PCR high-resolution melting (qPCR-HRM) analysis, a novel genotyping method. The plasma concentrations and risk of CHD were then analyzed in the northern Han Chinese population.

Table I. General characteristics and lipid levels in controls and CHD patients.

Characteristic	Control (n=417)	Cases (n=267)
Age (years)[b]	60.49±12.03	64.85±11.23
Female, n (%)	237 (56.83)	129 (48.31)
Body mass index (kg/m^2)	21.6±2.9	21.8±2.8
Non-smokers, n (%)[a]	261 (62.59)	126 (47.19)
Current smokers, n (%)[a]	141 (33.81)	132 (49.44)
Former smokers, n (%)	15 (3.60)	9 (3.37)
Non-alcohol, n (%)	336 (80.58)	187 (70.04)
Current alcohol, n (%)	72 (17.27)	73 (27.34)
Former alcohol, n (%)	9 (2.16)	6 (2.25)
Diabetes, n (%)[b]	48 (11.51)	74 (27.72)
Hypertension, n (%)	258 (61.87)	184 (68.91)
TC (mmol/l)	5.26±1.06	5.38±0.98
TG (mmol/l)	2.18±0.47	2.25±0.58
HDL-C (mmol/l)[a]	1.34±0.27	1.25±0.30
LDL-C (mmol/l)	3.27±0.72	3.34±0.71

[a]P<0.05, [b]P<0.01 vs. the control. TC, total cholesterol; TG, triglyceride; HDL-C, high density lipoprotein-cholesterol; LDL-C, low density lipoprotein-cholesterol; CHD, coronary heart disease.

Materials and methods

Study population. A total of 684 patients (417 with CHD and 267 healthy controls) were recruited from the Department of Cardiology, The Fourth Hospital of Jilin University (Changchun, Jilin, China). Informed consent was obtained from each patient included in the present study and ethical approval was provided by the Ethics Committee of The Fourth Hospital of Jilin University. The mean age of the study subjects was 62.67±11.63 years and the CHD patients were angiographically diagnosed as having CHD with 50% stenosis in one or more arteries and stable or unstable angina. The healthy controls were free of any clinical symptoms (including ischemia or asymptomatic myocardial infarction as determined by exercise electrocardiography or 24 h ambulatory electrocardiography monitoring). The present study was approved by the institutional review board of The Fourth Hospital of Jilin University and all participants signed a consent document prior to participation.

Data information. All participants were personally interviewed by our participating physicians and the initial clinical data, including age, sex, ethnicity, tobacco smoking status and alcohol consumption were recorded. The subjects were categorized into smokers (current smokers and former smokers), non-smokers and drinkers (current drinkers and former drinkers) and non-drinkers. Diabetes mellitus was diagnosed as a fasting plasma glucose of >7.0 mmol/l or a 120 min plasma glucose of >11.1 mmol/l, or by current use of insulin or hypoglycemic drugs. Hypertension was defined as having a systolic blood pressure ≥140 mmHg and/or a diastolic blood pressure ≥90 mmHg, and/or by current use of antihypertensive medications.

DNA extraction and real-time PCR. All study subjects donated 1 ml of venous blood that was collected in an anticoagulant tube through vein-puncture following an overnight fast of 12 h. These blood samples were centrifuged at 2,000 x g for 20 min at 4˚C and then aliquoted and stored immediately at -86˚C until use. For genotyping of these single nucleotide polymorphisms (SNPs), genomic DNA was extracted using a DNeasy blood and tissue kit (Qiagen, Valencia, CA, USA). DNA yield and purity were determined by the A260/A280 ratio with a Nanodrop 1000 spectrophotometer (Nanodrop Technologies, Wilmington, DE, USA).

For optimal real-time qPCR-HRM genotyping analysis, primers of the target loci (Table II) were designed using the freely available software Primer-BLAST (http://www.ncbi.nlm.nih.gov/tools/primer-blast) and these primers were synthesized by Invitrogen Life Technologies (Shanghai, China).

For real-time PCR, mixtures of 1 μl of 10X PCR buffer, 1 μl of 25 mM $MgCl_2$, 0.25 μl of 2.5 mM dNTPs, 0.5 μl of 20X Eva-green saturation dye, 0.25 μl of each forward and reverse primer (10 μmol/l), 1 μl of template DNA (10 ng/μl), 0.1 μl of 5U Taq enzyme and 5.65 μl of nuclease-free water, were added in triplicate to a 96-well PCR plate and amplified in a CFX96 real-time PCR system (Bio-Rad, Hercules, CA, USA). The cycling parameters of PCR were as follows: an initial cycle

Table II. Primer sequences for genotyping of ABCG5/8 SNPs using qPCR-HRM analysis.

SNP	Primer sequence	Allele
ABCG5 rs4131229	5'-GCTTGCTTGGAGGCATCTTG-3'	C
	5'-CGGTGGGTTTACCTGTGGC-3'	T
ABCG8 rs4148211	5'-TCTCCTCTGAAAGTGACAACAGC-3'	A
	5'-CCTTGAACCCAGGCGTG-3'	G
ABCG8 rs4148217	5'-CCGAGTCCTACGAAGATGCC-3'	A
	5'-GGGGGGCGGGTTCAGT-3'	C
ABCG8 rs3806471	5'-CATGGGGCCCACAGGTCT-3'	C
	5'-CTGCTCCAGGAAACAGAGTGAA-3'	A

qPCR-HRM, quantitative PCR high-resolution melting; SNPs, single nucleotide polymorphisms.

at 95˚C for 5 min, followed by 50 cycles of 95˚C for 10 sec, 60˚C for 15 sec and 72˚C for 25 sec. Following qPCR, DNA genotyping was performed using a high-resolution melting curve i.e. 95˚C for 1 min and 40˚C for 1 min. Fluorescence data were then collected ~40 times for every degree change during warm-up from 60-90˚C. The samples were then cooled at 40˚C for 10 sec. As an example, the amplification curve and typing results for s4148217 (A/C) are shown in Fig. 1. DNA samples detected by the qPCR-HRM were also confirmed by direct DNA sequencing (each genotype in five samples). DNA sequences were then analyzed using an ABI PRISM 3100 Genetic Analyzer sequencer (PE Biosystems, Foster City, CA, USA).

Laboratory analysis of serum lipid levels. All laboratory data were determined using fasting sera without any lipid-lowering drug usage for at least 4 weeks. Lipid levels were measured using an Hitachi 7170 automatic biochemical analyzer (Hitachi, Tokyo, Japan). Total cholesterol and triglyceride were measured by an enzyme method with reagents supplied by The DiaSys Diagnostic System Limited Company (Shanghai, China). low density lipoprotein-cholesterol (LDL-C) and high density lipoprotein-cholesterol (HDL-C) levels were detected by a direct enzyme method using reagents provided by Beijing Jiuqiang Biological Ltd. (Beijing, China) according to the manufacturer's instructions.

Statistical analysis. Epidemiological data were collected and analyzed against SNP and laboratory data using SPSS 17.0 (SPSS, Inc., Chicago, IL, USA). The baseline characteristics of participants were presented as the mean ± SD or as percentages of the study population. The differences in normal variables were analyzed using the Student's t-test (for categorical variables) and chi-square test or Fisher's exact test (for continuous variables). The Hardy-Weinberg equilibrium was assessed by the chi-square test. Genotype and allele frequencies for polymorphisms were calculated through direct counting. The chi-square test was used for genotype distributions and allele frequencies comparison. The impact of ABCG5/8 variants was analyzed in a dominant genetic model (heterozygous and homozygous for minor allele carriers were combined and compared with carriers of the common variant). To assess differences in plasma cholesterol concentrations among genotypes, the Chi-square test was performed. A multiple logistic regression analysis was used to evaluate the impact of ABCG5/8 gene polymorphisms on CHD and adjusted for significant risk factors by using an analysis of covariance. The results are expressed as odds ratios (OR) and 95% CI. P<0.05 was considered to indicate a statistically significant difference.

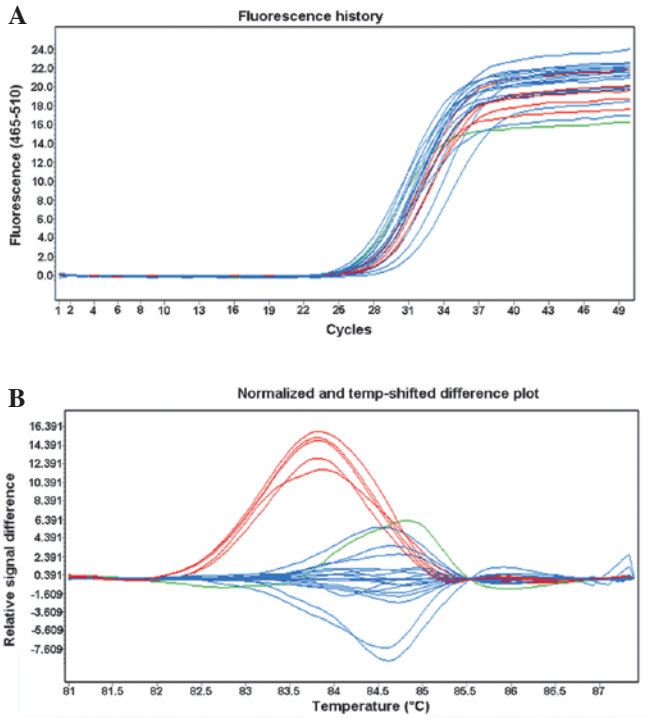

Figure 1. (A) Representative qPCR-HRM data set on the melting curves and genotype graphs of rs4148217. (A) Melting curve; (B) genotype graph. AA, green curve; CC, blue curve; AC, red curve. qPCR-HRM, quantitative PCR high-resolution melting.

Results

Characteristics of the study population. The characteristics of all the participants are shown in Table I. Among these variables, there were significant differences in age (P<0.05), tobacco smoking (P<0.05), HDL-C (P<0.05) and diabetes (P<0.01) between CHD patients and the healthy controls.

Table III. Genotype distribution and allele frequencies of ABCG5/8 polymorphisms.

		i7892T>C (rs4131229)		Tyr54CysA>G (rs4148211)		Thr400LysC>A (rs4148217)		5U145A>C (rs3806471)	
	SNP	Control	Case	Control	Case	Control	Case	Control	Case
N		417	261	417	261	417	261	417	261
Genotype, n (%)	AA	294 (70.50)	186 (71.26)	288 (69.06)	207 (79.31)	297 (71.22)	219 (83.91)*	288 (69.06)	201 (77.01)
	AB	114 (27.34)	72 (27.59)	120 (28.78)	48 (18.39)	114 (27.34)	36 (13.79)*	123 (29.50)	57 (21.84)
	BB	9 (2.16)	3 (1.15)	9 (2.16)	6 (2.30)	6 (1.44)	6 (2.30)	6 (1.44)	3 (1.15)
MAF (%)	A	15.83	14.94	16.55	11.49	15.10	9.20	16.19	12.07
N		141	126	141	126	141	126	141	126
Smokers	AA	99 (70.21)	87 (69.05)	102 (72.34)	99 (78.57)	195 (70.65)	117 (86.67)[a]	99 (70.21)	96 (76.19)
	AB+BB	42 (29.79)	39 (30.95)	39 (27.66)	27 (21.43)	81 (29.35)	18 (13.33)[a]	42 (29.79)	30 (23.81)
		276	135	276	135	276	135	276	135
Non-smokers	AA	195 (70.65)	99 (73.33)	186 (67.39)	108 (80.00)	102 (72.34)	102 (80.95)	189 (68.48)	105 (77.78)
	AB+BB	81 (29.35)	36 (26.67)	90 (32.61)	27 (20.00)	39 (27.66)	24 (19.05)	87 (31.52)	30 (22.22)

[a]P<0.05, control vs. case. Allele A, *rs4131229T*, *rs4148211A*, *rs4148217C* or *rs3806471A*. MAF, minor allele frequency; ABCG, ATP-binding cassette sub-family G.

Table IV. Univariate analyses of risk factors for CHD.

	Age	Gender	Smokers	Drinker	Hypertension	Diabetes	TC (mmol/l)	TG (mmol/l)	HDL-C (mmol/l)	LDL-C (mmol/l)	i7892T>C	Tyr54 CysA>G	Thr400 LysC>A	5U145 A>C
P-value	0.008	0.270	0.031	0.090	0.232	0.003	0.397	0.594	0.023	0.453	0.903	0.093	0.032	0.196
OR	1.033	1.353	1.827	1.737	1.411	2.929	0.889	0.961	0.265	0.869	1.028	1.717	2.107	1.501
95% CI	1.008-1.057	0.791-2.316	1.056-3.160	0.917-3.291	0.803-2.482	1.452-5.907	0.678-1.167	0.831-1.112	0.084-0.832	0.601-1.254	0.573-1.852	0.913-3.228	1.068-4.157	0.811-2.777

CHD, coronary heart disease; CI, confidence interval; OR, odds ratio; TC, total cholesterol; TG, triglyceride; HDL-C, high density lipoprotein-cholesterol; LDL-C, low density lipoprotein-cholesterol.

Table V. Association of ABCG8_ Thr400LysC>A with CHD risk by subgroup analyses.

Genotype		All participants	Tobacco smoke	Non-smokers	Male	Female	Alcohol drinking	Non-drinkers
Thr400Lys C>A (allele)	OR (95%CI)	2.107 (1.068-4.157)	2.700 (1.024-7.120)	1.625 (0.597-4.421)	2.059 (0.774-5.476)	2.108 (0.816-5.447)	2.722 (0.612-12.101)	1.926 (0.895-4.143)
	P-value	0.032	0.045	0.342	0.148	0.124	0.188	0.094
Adjusted log-dominant model	OR (95%CI)	2.034 (0.983-4.207)	2.663 (0.973-7.286)	1.472 (0.467-4.640)	2.220 (0.746-6.608)	1.975 (0.717-5.439)	2.152 (0.359-12.914)	1.972 (0.873-4.454)
	P-value	0.048	0.049	0.509	0.152	0.188	0.402	0.102

CI is adjusted for age, smoking, diabetes and HDL-C. CHD, coronary heart disease; OR, odds ratio; CI, confidence interval; HDL-C, high density lipoprotein-cholesterol; ABCG, ATP-binding cassette sub-family G.

Table VI. Association of ABCG5/8 polymorphism with plasma lipid levels in different genotypes.

		TC		TG		HDL-C		LDL-C	
SNP	Genotype	Control	Case	Control	Case	Control	Case	Control	Case
i7892T>C	TT	5.224±1.019	5.404±0.925	2.022±0.457	2.372±0.459	1.335±0.269	1.263±0.330	3.281±0.824	3.370±0.748
	TC+CC	5.306±1.102	5.352±1.029	2.195±0.486	2.130±0.697	1.349±0.267	1.239±0.271	3.260±0.615	3.311±0.665
Tyr54CysA>G	AA	5.271±1.050	5.388±0.922	2.129±0.462	2.302±0.576	1.322±0.279	1.241±0.328	3.268±0.772	3.351±0.747
	AG+GG	5.259±1.071	5.368±1.032	2.088±0.481	2.200±0.580	1.362±0.257	1.261±0.273	3.273±0.667	3.330±0.666
Thr400LysC>A	CC	5.280±1.048	5.432±1.032	2.139±0.464	2.534±0.460*	1.336±0.278	1.263±0.320	3.287±0.734	3.361±0.735
	CA+AA	5.250±1.073	5.324±0.922	2.078±0.479	1.968±0.696	1.348±0.258	1.239±0.281	3.254±0.705	3.320±0.678
5U145A>C	AA	5.210±0.998	5.390±0.927	1.992±0.439	2.330±0.512	1.349±0.276	1.248±0.321	3.291±0.797	3.335±0.738
	AC+CC	5.320±1.123	5.366±1.027	2.225±0.504	2.172±0.644	1.335±0.260	1.254±0.280	3.250±0.642	3.346±0.675
Smokers									
Thr400LysC>A	CC	5.293±1.173	5.382±1.028	2.300±0.515	2.712±0.586[a]	1.307±0.182	1.231±0.394	3.279±0.864	3.447±0.634
	CA+AA	5.214±0.992	5.246±1.025	1.934±0.486	1.998±0.575	1.324±0.262	1.225±0.276	3.357±0.519	3.411±0.665
Non-smokers									
Thr400LysC>A	CC	5.281±1.081	5.361±1.023	2.208±0.452	2.256±0.663	1.378±0.383	1.288±0.280	3.241±0.793	3.274±0.666
	CA+AA	5.132±0.996	5.491±0.832	1.992±0.433	2.038±0.488	1.359±0.245	1.260±0.252	3.206±0.702	3.230±0.861

All values are presented as the mean ± SD. [a]P<0.05 between groups. ABCG, ATP-binding cassette sub-family G; TC, total cholesterol; TG, triglyceride; HDL-C, high density lipoprotein-cholesterol; LDL-C, low density lipoprotein-cholesterol.

However, no significant difference was observed for gender, alcohol consumption, hypertension status or levels of total cholesterol (TC), triglyceride (TG) and LDL-C between the cases and controls (P>0.05 for all).

Distribution of genotype and allele frequencies in CHD patients and controls. Genotypic and allelic frequencies of candidate ABCG5/8 SNPs are shown in Table III. In particular, the genotype distributions observed for these four SNPs were consistent with the Hardy-Weinberg equilibrium (P>0.05). Among the SNPs examined, only the genotypic and allelic frequencies of ABCG8_Thr400LysC>A were significantly different (P<0.05) between the CHD patients (CC-83.91%, CA-13.79% and AA-2.30%; A=9.20%) and the healthy controls (CC-71.22%, CA-27.34% and AA-1.44%; A=15.10%). By contrast, no significant differences in genotype frequencies of the remaining three SNPs were identified between cases and controls (P>0.05 for all).

Association between ABCG5/8 polymorphism and susceptibility to CHD following adjusting for established risk factors. A stepwise multiple regression analysis was used to identify the genetic and environmental risk factors for CHD (Table IV). Age (OR=1.033; 95% [CI]=1.008-1.057; P=0.008), tobacco smoking (OR=1.827; 95% [CI]=1.056-3.160; P=0.031), and diabetes (OR=2.929; 95% [CI]=1.452-5.907; P=0.003) were major environmental risk factors for CHD in the studied population, while the levels of plasma HDL-C were a potent protective factor (OR=0.265; 95% [CI]=0.084-0.832; P=0.023) against CHD.

Binary logistic regression analysis of the four genetic variants studied revealed that CC subjects of the ABCG8_Thr400LysC>A SNP had a significant risk of developing CHD following adjusting for established risk factors (adjusted OR=2.304; 95% [CI]=0.983-4.207; P=0.048; Table V) compared with A allele carriers. The minor allele carriers of Tyr54CysA>G and 5U145A>C were also at an increased risk of CHD, however, the association was not statistically significant (Table IV).

Interaction between ABCG5/8 polymorphism and tobacco smoking in the development of CHD. Subgroup analyses of interactions between ABCG5/8 and tobacco smoking were performed and their impacts on the risk of CHD were assessed. The dominant model analysis demonstrated that among non-smokers, the genotype frequency of ABCG8_Thr400LysC>A SNP in CHD patients (CC=86.67%; CA + AA=13.13%) was significantly different from that in healthy controls (70.65 vs. 29.35; P=0.024; Table III). However, no significant differences in gender and alcohol consumption between cases and controls were identified (data not shown).

Furthermore, a stepwise multiple regression analysis revealed that CC subjects of the ABCG8_Thr400LysC>A SNP had a significantly increased risk of CHD in non-smokers (OR=2.663; 95% CI=0.973-7.286; adjusted P=0.049) compared with the A allele carriers. The ORs were adjusted by age, gender, history of diabetes mellitus and HDL-C level. However, no significant interaction between gender and alcohol consumption leading to a risk of CHD was identified (Table V).

Association of ABCG5/8 polymorphism with plasma lipid level in different genotypes. TG levels in carriers of the minor A allele of the ABCG8_Thr400LysC>A SNP were significantly lower than that of the homozygous CC (P=0.017; Table VI). CHD patients carrying the minor A allele of the ABCG8_Thr400LysC>A SNP had significantly lower baseline TG levels than CC homozygotes (P<0.05). However, no significant difference was observed in TC, LDL-C and HDL-C levels among the remaining genotypes in the two cases and controls (P>0.05).

In order to determine the effect of the interaction between ABCG5/8 and tobacco smoking on lipid levels, further subgroup analyses were performed. The data demonstrated that in tobacco smokers, TG levels in carriers of the minor A allele of the ABCG8_Thr400LysC>A SNP were significantly lower than in CC homozygotes (P<0.05). However, no significant differences in lipid concentrations between CHD subjects and controls in non-smokers were identified.

Discussion

The present study assessed four *ABCG5* and *ABCG8* SNPs and serum lipid levels for an association with the risk of developing CHD or dyslipidemia. The present case-control study included 417 CHD patients and 267 controls. Our data demonstrated clear associations between variants of ABCG5/8 and serum lipid levels and the risk of CHD in the northern Han Chinese population. However, in these four candidate SNPs, only the genotype and allele frequencies of ABCG8_T400K SNP were significantly different between the CHD and healthy control groups. TG levels in the CC homozygotes were significantly greater in CHD patients than in healthy controls. Binary logistic regression analysis demonstrated that the homozygous C allele at Thr400LysC>A resulted in a more than two-fold greater risk of developing CHD compared with those who carried the A allele in a dominant model. Multivariate analysis supported that such an effect was independent of several other risk factors, including age, gender, history of diabetes mellitus and HDL-C level. Furthermore, tobacco smoking CC homozygotes at Thr400LysC>A had a 2.7-fold higher risk for CHD than either the CA or AA genotype. The current study did not provide evidence of an interaction between genotype and alcohol consumption in terms of CHD risk, and also did not find an association between genotype and gender of the subjects as previous studies have reported in other ethnicities (16,24). However, a future study with a larger sample size is required to verify our current findings prior to using the information for CHD prevention in high-risk populations in a clinical setting.

At the cell biology level, ABCG5 and ABCG8 proteins are hemi-transporters that limit intestinal absorption, however, promote biliary excretion of sterols (8), which are localized on human chromosome 2p21, and are tandemly arrayed in a head-to-head orientation separated by 374 bp. The two proteins have 13 exons and 12 introns and span ~28 kb DNA sequences (10). To date, several SNPs have been identified in ABCG5 and ABCG8 (10,25). In the current study, rs4131229 for ABCG5 and rs4148211, rs4148217 and rs3806471 for ABCG8 were selected. However, rs11887534 and rs6544718 were not included in the present study as they were rare variants in the Chinese popu-

lation (i.e. minor allele frequency <1%) (26). Several previous studies demonstrated that the ABCG8 Thr400LysC>A SNP may have an ethnic specificity (15,27-30). In the present study, the genotype distribution of these four variants in Northern Han Chinese participants deviated slightly from expectations of the Hardy-Weinberg equilibrium (P>0.05). For example, frequencies of CC, CA and AA at ABCG8_Thr400LysC>A SNP were 83.91, 13.79 and 2.30%, respectively in the CHD group, while they were 71.22, 27.34 and 1.44%, respectively in the healthy controls, which were similar to a previous study on Chinese patients with gallstones (CC, CA and AA genotypes were 83.1, 16.4 and 0.5%, respectively) (31). The frequencies observed in the present study were different from those in the Boston Puerto Rican Health cohort (27) (frequencies of CC and CA/AA genotypes were 60.0 and 40.0%, respectively), a German study (15) on gallstones (frequencies of CC and CA/AA genotypes were 58.3% and 41.7%, respectively) and a Czech study (18) (frequencies of CC, CA and AA genotypes were 65.4, 31.3 and 3.3%, respectively). These genetic diversities among various ethnicities may result from the consumption of different food, dietary history and other environmental factors, indicating gene-environment interactions that alter human gene activities and the risk of disease development. Thus, genetic diversity may also produce potentially contradictory polymorphisms between genes and disease risk in different ethnic groups.

In the current study in a northern Han Chinese population, the homozygous CC ABCG8 Thr400LysC>A SNP carriers exhibited significantly higher TG levels than the A allele carriers. This conclusion corroborates the findings of a previous study, which demonstrated that females with the A allele had lower plasma TG than C homozygotes (31). Several other studies have also demonstrated a significant association between baseline TG levels and the p.T400K polymorphism (16,32,33). Notably, several association studies have also demonstrated that there is no significant association between the T400K polymorphism and plasma levels of TG (28,29,34,35). A study of siblings with gallstones (36) demonstrated that male ABCG8 CC homozygotes had greater decreases in TG and LDL-C levels than CA/AA carriers following dietary alteration. Furthermore, neither ABCG8 T400K nor D19H polymorphisms are independently associated with the risk of CHD in a cohort containing 2,012 heterozygous FH patients (37). Another study suggests that ABCG5/G8 polymorphisms are not associated with the risk of CHD (38). An additional study suggests that this locus affects risk of CHD either directly through its effect on plasma phytosterol levels or through primary/secondary changes in LDL-cholesterol (39). Notably, ABCG8 alleles at rs41360247 are associated with reduced phytosterol levels, thus indicating further association with reduced risk of CHD (40). However, to date, to the best of our knowledge, there has been no study that linked these SNPs to CHD in the Han Chinese population. In the current study, ABCG8 CC homozygotes had >two-fold risk of CHD compared with A allele carriers, which may imply that ABCG8 T400K variants are a novel gene marker for CHD risk in the northern Han Chinese population. Indeed, the T400K variant is a missense mutation that changes the amino acid residue at position 400 from arginine to histidine in ABCG8. Nationally representative surveys in China indicate that the long-term nutritional trend of Chinese has shifted towards a Westernized lifestyle, i.e. a high-fat, high-energy and low-fiber diet. Several studies have indicated that dietary constituents perturb cholesterol homeostasis and consequently affect expression of hepatic transporters. Diets rich in cholesterol and cholic acid reportedly enhance the expression of ABCG5/8 in the liver (41,42). Hubacek *et al* (18) suggested that the Thr400 allele may be a 'diet-responsive' allele and a long-term change in dietary composition may affect the expression of ABCG5/8 genes. Thus, it may be expected that the expression of the ABCG5/8 gene, in C allele carriers at Thr400Lys, is able to increase markedly while consuming a high fat diet to limit cholesterol absorption but promote hepatic secretion.

Cigarette smoke is an established risk factor for developing CHD and is considered as one of the major risk factors for the development of atherosclerosis disease (43). A previous study suggested that smokers who are carriers of the ABCG8 Thr400 allele exhibit lower HDL-C concentrations and thereby have a potential increased risk for atherosclerosis (37), while smokers who carry the minor alleles at ABCG5 (i7892A>G, i18429C>T or i11836G>A) SNPs exhibit significantly lower HDL-C, higher TC and higher TG, respectively. In the current study, the dominant model analysis demonstrated that tobacco smokers, CC-homozygous at the ABCG8_Thr400LysC>A SNP, had increased TG levels and risk of CHD compared with CA/AA tobacco smokers. The present study hypothesized that tobacco smoke may lower the transcription of ABCG5/8 and lead to intracellular cholesterol accumulation, development of CHD and premature atherosclerosis. However, the current study had a relatively small sample size and did not allow us to conduct haplotype analyses. Further functional analyses of the variants examined in the present study have not yet been conducted. Additionally, further study is required in order to elucidate the mechanisms of ABCG5/8 variants on CHD development in the Han Chinese population.

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