The effect of sterol regulatory element-binding protein 2 polymorphism on the serum lipid in northern Chinese subjects

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Abstract Sterol regulatory element-binding protein 2 (SREBP-2) is an important nuclear transcription factor in the regulation of cellular cholesterol metabolism. To determine allele frequency of the 1784G>C polymorphism at the SREBP-2 locus and investigate the relationship between this polymorphism and serum lipid levels in Chinese people, we selected 486 individuals (118 men and 368 women) from the Xicheng District of Beijing. The subjects were divided into four groups: hypercholesterolemic subjects, hypertriglyceridemic subjects, combined hyperlipidemic subjects, and normal subjects. Serum lipid profiles were measured in all subjects, and 1784G>C was analyzed using polymerase chain reaction–restriction fragment length polymorphism analysis. There was no significant difference in genotype frequencies or allele frequencies of this polymorphism between the hyperlipidemic and control groups. The serum total cholesterol (TC) and LDL cholesterol (LDL-C) levels of the individuals carrying the C allele were higher than the noncarriers in both males and females in the hypercholesterolemic group, but statistical significance was only observed in females. The results of this study indicate that the SREBP-2 polymorphism is related to elevated concentrations of serum TC and LDL-C in hypercholesterolemic subjects. Further work is necessary to confirm the role of 1784G>C in the development of hyperlipidemia.—Duan, X., W. Zhu, Y. Li, Z. Zhang, Y. Zhao, J. Dao, and Y. Xiao. **The effect of sterol regulatory element-binding protein 2 polymorphism on the serum lipid in northern Chinese subjects.** ***J. Lipid Res.*** **2005.** 46: **252–257.**

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Hyperlipidemia is an important risk factor for atherosclerosis and premature coronary artery disease, which is one of the major contributors to mortality in the world. With the increasing recognition of genetic–environmental interactions in the etiology of hyperlipidemia and the important advances in DNA technology, it is necessary to include genetic information in epidemiological studies to identify how some polymorphic traits are associated with several phenotypic traits. However, our knowledge about the genetic factors determining lipid profiles in the general population is still limited (1–3). Recent studies have shown that genetic alterations of sterol regulatory element-binding proteins (SREBPs) can contribute to disorders of lipid metabolism.

Since SREBPs were purified in 1993 from nuclear extracts of cultured human Hela cells, their roles have been established as lipid synthetic transcription factors, especially in cholesterol and fatty acid synthesis (4). SREBPs, which are embedded in the membranes of the endoplasmic reticulum, consist of four domains: two membrane-spanning regions and an amino- and carboxyl-terminal portion that project into the cytoplasm (5–8). The *N*-terminal domain of approximately 480 amino acids is a transcription factor of the basic helix-loop-helix leucine zipper family. When cells are deprived of cholesterol, SREBPs are cleaved continuously by two proteases (S1P and S2P) that release the NH_2-terminal, creating mature forms of the SREBPs that enter the nucleus and bind to the specific DNA sequence in the promoters of their target genes (e.g., the LDL receptor and HMG-CoA synthase). This cleavage is inhibited when cells are overloaded with sterols. Thus, cleavage activation of SREBPs can increase LDL receptor–mediated plasma cholesterol uptake and cholesterol biosynthesis (9–17).

To date, three members of the SREBP family have been identified: SREBP-1a, SREBP-1c, and SREBP-2 (18–21). Evidence from various studies indicates that SREBP-2 is more specific to cholesterogenic genes containing SREs in their promoters, whereas SREBP-1 is more active for lipogenic genes including E-boxes (8). SREBP-2 is encoded by a separate gene on human chromosome 22q13, which

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Abbreviations: LDL-C, low density lipoprotein cholesterol; SCAP, SREBP cleavage-activating protein; SREBP-2, sterol regulatory element-binding protein 2; TC, total cholesterol; TG, triglyceride.

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TABLE 1. Characteristics (mean [95% confidence interval]) of the study population

Variable	Males (n = 118) HC (n = 18)	HT (n = 32)	CHL (n = 25)	CG (n = 43)	P HC vs. CG	P HT vs. CG	P CHL vs. CG	Females (n = 368) HC (n = 88)	HT (n = 74)	CHL (n = 85)	CG (n = 121)	P HC vs. CG	P HT vs. CG	P CHL vs. CG
Age (years)	58.9 (53.8–64.0)	56.8 (53.3–60.3)	54.0 (50.7–57.3)	59.1 (56.4–61.8)	0.928	0.264	0.023	58.7 (57.1–60.3)	57.6 (56.0–59.2)	59.1 (57.7–60.6)	55.0 (53.5–56.6)	0.001	0.026	<0.001
BMI (kg/m^2)	25.5 (24.3–26.8)	27.3 (26.4–28.1)	28.8 (27.5–30.1)	26.0 (25.0–27.0)	0.575	0.087	0.001	27.3 (26.5–28.1)	28.8 (27.9–29.6)	28.3 (27.5–29.1)	26.6 (25.9–27.4)	0.223	<0.001	0.003
Fasting glucose (mmol/l)	5.25 (4.58–5.93)	5.91 (4.93–6.89)	6.57 (5.54–7.60)	5.34 (4.86–5.83)	0.873	0.240	0.019	5.43 (4.99–5.87)	5.90 (5.52–6.29)	6.79 (6.15–7.44)	5.01 (4.77–5.25)	0.137	0.003	<0.001
SBP (mm Hg)	142.7 (133.2–152.2)	142.3 (136.5–148.0)	138.0 (131.0–145.0)	141.7 (133.7–149.7)	0.864	0.913	0.485	133.9 (130.4–137.5)	141.9 (136.9–146.8)	139.3 (135.1–143.4)	132.4 (128.6–136.1)	0.574	0.001	0.014
DBP (mm Hg)	90.2 (85.6–94.9)	92.4 (88.2–96.6)	91.3 (86.6–96.1)	88.2 (84.3–92.1)	0.547	0.133	0.297	85.0 (82.7–87.2)	88.8 (86.0–91.5)	87.7 (85.5–90.0)	84.7 (82.7–86.6)	0.843	0.013	0.052
TG (mmol/l)	1.18 (1.05–1.32)	2.69 (2.18–3.20)	3.69 (2.89–4.49)	1.07 (0.97–1.18)	0.727	<0.001	<0.001	1.23 (1.17–1.29)	2.42 (2.25–2.59)	2.99 (2.65–3.32)	1.03 (0.98–1.08)	0.074	<0.001	<0.001
TC (mmol/l)	6.38 (6.08–6.68)	5.09 (4.92–5.26)	6.75 (6.39–7.10)	4.45 (4.22–4.68)	<0.001	<0.001	<0.001	6.27 (6.14–6.40)	5.05 (4.94–5.16)	6.52 (6.39–6.65)	4.52 (4.44–4.60)	<0.001	<0.001	<0.001
HDL-C (mmol/l)	1.47 (1.36–1.58)	1.10 (1.02–1.18)	1.25 (1.14–1.36)	1.33 (1.27–1.39)	0.030	<0.001	0.144	1.58 (1.52–1.63)	1.19 (1.13–1.24)	1.31 (1.26–1.36)	1.45 (1.41–1.48)	<0.001	<0.001	<0.001
LDL-C (mmol/l)	4.37 (4.04–4.70)	2.76 (2.50–3.03)	3.82 (3.41–4.23)	2.73 (2.58–2.87)	<0.001	0.828	<0.001	4.14 (4.01–4.26)	2.77 (2.64–2.89)	3.85 (3.71–4.00)	2.60 (2.53–2.68)	<0.001	0.037	<0.001
Apolipoprotein A1 (g/l)	1.27 (1.25–1.30)	1.27 (1.23–1.31)	1.29 (1.26–1.32)	1.25 (1.23–1.27)	0.269	0.176	0.022	1.25 (1.23–1.26)	1.24 (1.23–1.26)	1.27 (1.25–1.29)	1.23 (1.22–1.25)	0.148	0.326	0.001
Apolipoprotein B (g/l)	0.96 (0.93–1.00)	0.88 (0.85–0.92)	1.08 (1.03–1.12)	0.75 (0.73–0.78)	<0.001	<0.001	<0.001	0.94 (0.92–0.96)	0.86 (0.84–0.87)	1.02 (1.00–1.04)	0.71 (0.69–0.73)	<0.001	<0.001	<0.001
Smokers	16.7%	53.1%	40.0%	32.6%		0.009		12.5%	9.5%	9.4%	8.3%		0.124	
Drinkers	27.8%	31.3%	56.0%	32.6%		0.147		5.7%	4.1%	5.9%	1.7%		0.375	

BMI, body mass index; CG, control group; CHL, combined hyperlipidemia (total cholesterol ≥5.72 mmol/l and triglyceride ≥1.70 mmol/l); DBP, diastolic blood pressure; HC, hypercholesterolemia (total cholesterol ≥5.72 mmol/l and triglyceride <1.70 mmol/l); HDL-C, HDL cholesterol; HT, hypertriglyceridemia (total cholesterol <5.72 mmol/l and triglyceride ≥1.70 mmol/l); LDL-C, LDL cholesterol; SBP, systolic blood pressure; TC, total cholesterol; TG, triglyceride. TG was log-transformed for the analysis and back to obtain the mean. *P* values were obtained in the comparison among the four groups studied (ANOVA for means and chi-square test for percentages).

spans 72 kb and is composed of 19 exons and 18 introns (22, 23). Several polymorphisms and rare variants in this gene have been detected, and some of these have been shown to have a significant effect on serum lipid levels (24, 25). A common polymorphism detected using *Msp 1* is a G-to-C transversion at nucleotide position 1784 of the SREBP-2 cDNA. The presence of 1784C abolishes a *Msp 1* restriction site and causes a glycine-to-alanine substitution (G595A). This 1784G>C polymorphism (G595A) was reported to be associated with serum levels of total cholesterol (TC) in hypercholesterolemic subjects from Switzerland and Israel (12). However, this association might be population-specific and highly influenced by environmental factors. Therefore, the aim of this study was to estimate allele frequencies of 1784G>C and to investigate the relationship between this polymorphism and serum lipid levels in hyperlipidemic and normolipidemic individuals residing in Beijing, China. The region presents a relatively high rate of hyperlipidemia. The results of this research could contribute to our understanding of the genetic factors associated with hyperlipidemia risk.

TABLE 2. Genotype distribution and allele frequencies of the 1784G>C polymorphism by sex and serum lipid status in a Chinese population

Group	Genotype Frequencies GG	GC	CC	Allele Frequencies G	C	P
Male						
HC (n = 18)	61.1 (11)	38.9 (7)	0 (0)	80.5	19.5	0.567
HT (n = 32)	71.9 (23)	25.0 (8)	3.1 (1)	84.4	15.6	
CHL (n = 25)	76.0 (19)	24.0 (6)	0 (0)	88.0	12.0	
CG (n = 43)	69.8 (30)	23.2 (10)	7.0 (3)	81.4	18.6	
Female						
HC (n = 88)	65.9 (58)	28.4 (25)	5.7 (5)	80.1	19.9	0.996
HT (n = 74)	60.8 (45)	32.4 (24)	6.8 (5)	77.0	23.0	
CHL (n = 85)	65.9 (56)	28.2 (24)	5.9 (5)	80.0	20.0	
CG (n = 121)	64.5 (78)	29.8 (36)	5.7 (7)	79.4	20.6	

CG, control group; CHL, combined hyperlipidemia (total cholesterol ≥5.72 mmol/l and triglyceride ≥1.70 mmol/l); HC, hypercholesterolemia (total cholesterol ≥5.72 mmol/l and triglyceride <1.70 mmol/l); HT, hypertriglyceridemia (total cholesterol <5.72 mmol/l and triglyceride ≥1.70 mmol/l). Values shown are frequency and (number of subjects). *P* values were obtained among the control group and HC, HT, and CHL groups across 1784G>C genotypes by sex using the chi-square test.

MATERIALS AND METHODS

Subjects

The study was conducted in eight community clinics in the Xicheng District of Beijing from March to September 2001. A total of 486 unrelated individuals (118 men and 368 women) between the ages of 30 and 80 years (mean: 58 years) were randomly selected. Four groups were considered: normal subjects, hypercholesterolemic subjects, hypertriglyceridemic subjects, and combined hyperlipidemic subjects. None of the subjects had gastrointestinal, thyroid, liver, or renal diseases or diabetes mellitus, and none was treated with a lipid-lowering diet or drugs at the time of blood sampling for determination of lipoprotein parameters.

The study protocol was approved by the Ethical Committees of the Institutions, and informed consent was obtained from each participant.

Lipid and lipoprotein measurements

Blood samples were collected after overnight (>12 h) fasting. Serum TC and high-density lipoprotein cholesterol (HDL) were measured by way of cholesterol oxidase-p-aminophenazone (CHOD-PAP). Triglycerides (TGs) were measured using the glycerol-3-phosphate oxidase-p-aminophenazone (GPO-PAP) method (26, 27). Apolipoprotein A-1 and apolipoprotein B were analyzed using the immunoturbidimetric end point method. LDL cholesterol (LDL-C) was calculated according to Friedewald's formula.

DNA analysis

Genomic DNA was extracted from white blood cells via phenol and chloroform extraction and ethanol precipitation essentially following standard protocols. A fragment of 247 bp in exon 10 of SREBP-2 was amplified via PCR for identification of 1784G>C. The PCR reaction was performed in a total volume of 25 μl, containing 2.5 μl of amplification buffer (100 mM Tris-HCl pH 9.0, 500 mM KCl, 15 mM $MgCl_2$ and 1.0% Triton X-100), 200 μM each nucleotide (dATP, dCTP, dGTP, and dGTP), 10 pmol of each primer (U: GCCAGTGACCATTAACACCTTTTGA; L: TCGTCTTCAAAGCCTGCCTCAGTGGCTGGC), 3 units of Taq DNA polymerase (Sino-American Biotechnology Co, Luoyang, Henan, China), and 0.5 μg of genomic DNA. The PCR conditions were 95°C for 5 min followed by 30 subsequent cycles at 94°C for 30 s, 55°C for 30 s, 72°C for 30 s, and 72°C for 2 min. PCR products were digested with 12 units of restriction endonuclease *MspI* at 37°C for 6 h in the buffer recommended by the manufacturer (Sino-American Biotechnology Co.). The restriction fragments were electrophoresed in 20% polyacrylamide gels at 90 V for 4 h and directly visualized using UV light after ethidium bromide staining of the acrylamide gels. All gels were reread blindly by two persons without any change, and 10% of the analyses were repeated randomly.

Statistical analysis

SPSS version 12.0 software (SPSS, Inc., Chicago, IL) was used for all analyses. The data were analyzed separately for each sex. A *P* value of less than 0.05 was considered statistically significant. Allele frequency was determined via direct counting, and the standard goodness-of-fit test was used to test the Hardy-Weinberg equilibrium for each sex. Differences in genotype distribution between the control group and the dyslipidemic groups were obtained using the chi-square test. Continuous variables were examined for the normality of their distribution. TGs were significantly skewed and were logarithmically transformed to improve normality. Statistical analysis of TGs was performed using transformed data. The mean and the confidence intervals presented for TGs corresponded to the antilog of the log-transformed values. ANOVA was used to compare mean differences for continuous variables among genotypes or among lipidemic groups. The influence of covariates in the comparison of means was con-

TABLE 3. Comparison of serum lipids by carrier status of 1784C mutation for males and females

	HC			HT			CHL			CG		
	GG	GC/CC	*P*	GG	GC/CC	*P*	GG	GC/CC	*P*	GG	GC/CC	*P*
Male												
TG (mmol/l)	1.16 (1.00–1.33)	1.22 (1.00–1.43)	0.687	2.79 (2.14–3.44)	2.43 (1.32–3.55)	0.594	3.40 (2.48–4.33)	4.60 (2.80–6.39)	0.254	1.11 (0.99–1.23)	0.99 (0.80–1.17)	0.267
TC (mmol/l)	6.21 (5.80–6.63)	6.63 (6.10–7.16)	0.211	5.20 (5.01–5.38)	4.81 (4.50–5.13)	0.051	6.87 (6.42–7.32)	6.36 (5.48–7.23)	0.315	4.31 (4.04–4.58)	4.78 (4.37–5.19)	0.064
HDL-C (mmol/l)	1.50 (1.35–1.65)	1.42 (1.23–1.61)	0.503	1.09 (0.99–1.19)	1.15 (0.98–1.32)	0.528	1.29 (1.15–1.42)	1.12 (0.86–1.38)	0.268	1.30 (1.23–1.37)	1.41 (1.29–1.52)	0.112
LDL-C (mmol/l)	4.19 (3.69–4.69)	4.66 (4.02–5.30)	0.238	2.84 (2.52–3.16)	2.56 (2.01–3.11)	0.392	4.04 (3.56–4.52)	3.15 (2.22–4.08)	0.109	2.65 (2.49–2.80)	2.91 (2.67–3.15)	0.074
Apolipoprotein A1 (g/l)	1.29 (1.26–1.33)	1.24 (1.20–1.29)	0.094	1.29 (1.24–1.33)	1.24 (1.16–1.32)	0.377	1.29 (1.26–1.32)	1.31 (1.25–1.38)	0.480	1.24 (1.22–1.26)	1.28 (1.24–1.31)	0.056
Apolipoprotein B (g/l)	0.95 (0.89–1.01)	0.99 (0.91–1.07)	0.385	0.89 (0.85–0.93)	0.87 (0.80–0.94)	0.647	1.09 (1.03–1.14)	1.04 (0.94–1.15)	0.474	0.74 (0.71–0.77)	0.79 (0.75–0.84)	0.044
Female												
TG (mmol/l)	1.23 (1.15–1.30)	1.24 (1.14–1.35)	0.815	2.50 (2.28–2.73)	2.29 (2.00–2.57)	0.250	2.92 (2.51–3.34)	3.10 (2.51–3.70)	0.634	1.03 (0.96–1.09)	1.04 (0.95–1.13)	0.834
TC (mmol/l)	6.13 (5.98–6.28)	6.55 (6.33–6.76)	0.002	5.02 (4.88–5.16)	5.10 (4.93–5.28)	0.447	6.52 (6.36–6.69)	6.52 (6.29–6.76)	0.997	4.50 (4.40–4.60)	4.56 (4.43–4.70)	0.437
HDL-C (mmol/l)	1.53 (1.46–1.59)	1.67 (1.59–1.76)	0.008	1.20 (1.13–1.27)	1.17 (1.08–1.26)	0.585	1.30 (1.24–1.36)	1.32 (1.24–1.41)	0.719	1.44 (1.40–1.48)	1.47 (1.41–1.52)	0.502
LDL-C (mmol/l)	4.05 (3.90–4.20)	4.30 (4.10–4.52)	0.050	2.69 (2.53–2.85)	2.90 (2.70–3.10)	0.111	3.89 (3.70–4.07)	3.79 (3.53–4.05)	0.547	2.59 (2.49–2.69)	2.63 (2.50–2.76)	0.670
Apolipoprotein A1 (g/l)	1.25 (1.23–1.27)	1.25 (1.23–1.28)	0.780	1.25 (1.23–1.27)	1.23 (1.21–1.26)	0.273	1.27 (1.25–1.29)	1.27 (1.24–1.30)	0.870	1.24 (1.22–1.25)	1.23 (1.21–1.26)	0.825
Apolipoprotein B (g/l)	0.94 (0.91–0.96)	0.95 (0.92–0.98)	0.362	0.85 (0.83–0.87)	0.87 (0.84–0.90)	0.193	1.03 (1.00–1.05)	1.00 (0.96–1.04)	0.331	0.72 (0.69–0.74)	0.70 (0.67–0.73)	0.394

CG, control group; CHL, combined hyperlipidemia (total cholesterol ≥5.72 mmol/l and triglyceride ≥1.70 mmol/l); HC, hypercholesterolemia (total cholesterol ≥5.72 mmol/l and triglyceride <1.70 mmol/l); HT, hypertriglyceridemia (total cholesterol <5.72 mmol/l and triglyceride ≥1.70 mmol/l). Values shown are the mean (95% confidence interval). *P* values were obtained in the comparison between GG and carriers of the C allele after multivariate adjustment (age, sex, body mass index, systolic blood pressure, diastolic blood pressure, fasting glucose, tobacco, and alcohol).

TABLE 4. Risk of different dyslipidemic groups according to the presence of the 1784C allele in a Chinese population and stratification by sex

		Total				Male				Female			
Gene Variant	Risk	n[a]	OR	95% CI	*P*	n[a]	OR	95% CI	*P*	n[a]	OR	95% CI	*P*
GG	(Reference)		1				1				1		
GC/CC	HC vs. CG	106/164	1.03	0.60–1.78	0.898	18/43	1.47	0.40–5.37	0.591	88/121	0.94	0.51–1.74	0.829
	HT vs. CG	106/164	1.08	0.63–1.86	0.774	32/43	0.90	0.29–2.77	0.843	74/121	1.17	0.62–2.22	0.608
	CHL vs. CG	110/164	0.90	0.52–1.55	0.688	25/43	0.73	0.20–2.55	0.581	85/121	0.96	0.50–1.75	0.833

CG, control group; CHL, combined hyperlipidemia (total cholesterol ≥5.72 mmol/l and triglyceride ≥1.70 mmol/l); CI, confidence interval; HC, hypercholesterolemia (total cholesterol ≥5.72 mmol/l and triglyceride <1.70 mmol/l); HT, hypertriglyceridemia (total cholesterol <5.72 mmol/l and triglyceride ≥1.70 mmol/l); OR, odds ratio.

[a] Number of HC, HT, or CHL group /number of control group.

trolled by multiple linear regression analyses. Lipid concentrations were adjusted for age, sex, body mass index, systolic blood pressure, diastolic blood pressure, fasting glucose, tobacco smoking, and alcohol intake. Because of the low numbers of individuals homozygous for the rare allele, they were pooled with heterozygotes and subsequently classified as carriers or noncarriers of the rare allele (1784C). To estimate the risk of dyslipidemia with less common allele, the odds ratio and 95% confidence interval were computed. Multivariate linear regression analysis with dummy variables for categorical terms was used to test the null hypothesis of association between genetic variants and lipid levels. Regression coefficients and the proportion of variance attributable to each predictor were estimated from the models.

RESULTS

Adjusted mean lipid levels for males and females are presented in **Table 1**. As expected, hypercholesterolemic, hypertriglyceridemic, and combined hyperlipidemic individuals had higher serum concentrations of TC, LDL-C, and TGs and lower HDL cholesterol concentrations than normolipidemic populations.

Frequencies of the 1784(G>C) polymorphism

Genotypes and allele frequencies in men and women for the four lipidemic groups are shown in **Table 2**. There was no significant deviation from Hardy-Weinberg equilibrium in each group. No significant differences in genotype frequencies or allele frequencies of 1784(G>C) were found.

SREBP-2 polymorphism and serum lipids

The assessment of associations between 1784C allele carrier status and lipid levels was performed for males and females after multivariable adjustment (**Table 3**). The serum TC and LDL-C levels of individuals carrying the C allele were higher than those of the noncarriers in both the male and female hypercholesterolemic groups, but statistical significances were only observed in females. The presence of the 1784C allele was also associated with higher HDL cholesterol in the female hypercholesterolemic group. There were no statistically significant differences for lipid and lipoprotein concentrations by sex across genotypes in the hypertriglyceridemic and combined hyperlipidemic groups.

The association between the SREBP-2 gene variants and lipid status was examined further. **Table 4** shows the estimation of the risk of hyperlipidemia according to the presence of the 1784C allele. The results were more inconsistent, and no statistically significant associations were detected.

To estimate the effects of genetic variants, several linear regression models were fitted. **Table 5** shows regression coefficients for each variable by combining men and women. After the adjustment for covariates, the association of the SREBP-2 variant with TC level remained statistically significant in hypercholesterolemic group. No statistically significant gene–sex interactions were observed for the SREBP-2 gene variants.

DISCUSSION

Hyperlipidemia—mainly the hypercholesterolemia caused by elevated serum concentrations of LDL-C and TC—is associated with a high incidence of coronary artery disease. Considering the central role of SREBP-2 in the regulation

TABLE 5. Association between 1784G>C polymorphism and total cholesterol levels in a Chinese population: multiple linear regression analysis

	TC (mmol/l)							
	HC		HT		CHL		CG	
	B (SE)	*P*	B (SE)	*P*	B (SE)	*P*	B (SE)	*P*
Polymorphism carriers of 1784C allele	0.373 (0.120)	0.003	−0.039 (0.143)	0.689	0.005 (0.139)	0.970	0.152 (0.091)	0.097
Body mass index (kg/m^2)	−0.172 (0.127)	0.178	0.136 (0.117)	0.248	−0.143 (0.171)	0.405	0.181 (0.088)	0.042
Nonsmoking vs. smoking	−0.137 (0.178)	0.443	−0.218 (0.132)	0.102	0.225 (0.185)	0.226	−0.077 (0.132)	0.562
Nondrinker vs. drinker	0.166 (0.212)	0.434	−0.405 (0.161)	0.014	0.451 (0.208)	0.032	0.072 (0.166)	0.616
Men vs. women	−0.033 (0.158)	0.834	−0.019 (0.115)	0.868	0.068 (0.189)	0.721	0.070 (0.109)	0.522
R^2 of the model	0.115	0.030	0.087	0.100	0.095	0.063	0.042	0.236

of lipid metabolism, common genetic alterations in this gene may also contribute to polygenic hyperlipidemia.

In this study, we have determined for the first time in a Chinese population the relative allele frequency of the 1784G>C polymorphism of the SREBP-2 gene. The frequency of the C allele (absence of *Msp1* cleavage site) in control group (0.201) was slightly lower than those found in normolipidemic individuals from Switzerland (0.24), Israel (0.30) (12), and France (0.26) (24). Thus no significant difference in allelic and genotypic frequencies among the four groups was observed. The genotype distributions for 1784G>C were similar to the ones expected in Hardy-Weinberg equilibrium.

The serum TC and LDL-C levels of the individuals carrying the C allele were higher than those of the noncarriers in both the male and female hypercholesterolemic groups, but statistical significances were only observed in females. There was no association between serum lipids and CC/CG genotypes in the normal, hypertriglyceridemic, and combined hyperlipidemic groups. The 1784G>C polymorphism (G595A) was also reported to have a significant impact on TC level in the hypercholesterolemic subjects from Switzerland and Israel (12). However, this effect was not found in normocholesterolemic individuals. On the other hand, Robinet et al. have demonstrated that there was only a trend between TC values and the 1784G>C polymorphism in a selected population of men with a cardiovascular risk from France when hypercholesterolemic and normocholesterolemic subjects were considered separately (24).

In the present study, as we expected, the SREBP-2 polymorphism may be one of the genetic determinants leading to elevated serum TC and LDL-C levels. The 1784G>C variant in the 10 extron of the SREBP-2 gene can cause a glycine-to-alanine substitution (G595A), which is located in the COOH terminal regulation region that directly interacts with the SREBP cleavage-activating protein (SCAP). The substitution of G595A could damage the formation and/or stability of the SCAP/SREBP-2 complex by changing the α-helical protein structure; as a result, the 595A isoform would be less efficient than 595G in the maturation of SREBP-2 in case of a sterol depletion of the cell. This weakened efficiency can cause a decreased activation of the LDL receptor-mediated uptake of cholesterol-containing particles from the plasma; consequently, plasma TC and LDL-C levels would be expected to increase (12, 24). In our study, the cholesterol-elevating effect of the 1784C allele was only found in the hypercholesterolemic group, not in other subgroups. This fact could be explained by a gene–gene or gene–environment interaction with little effect in the overall population, but with a relatively strong contribution to the variability of TC and LDL-C in hypercholesterolemia (12). Therefore, further studies are necessary to establish the association of this polymorphism to variation in lipid concentrations in a larger population.

Recent studies have also shown that 1784G>C polymorphism of SREBP-2 gene and −36delG polymorphism of SREBP-1a gene can contribute to the development of atherosclerosis. Both two SREBPs polymorphisms may directly cause subtle dysregulation of the intracellular cholesterol metabolism without effects on plasma lipid levels (12, 28).

In addition to the 1784G>C variant, more than five rare mutations have been identified in the SREBP-2 gene to date, and none of the lipid parameters has been associated with these polymorphisms (e.g., 1668G>T, 3474T>C, 3705C>T, etc.).

In summary, the 1784G>C polymorphism may be a good genetic marker for hypercholesterolemia, and the study of this gene–environmental interaction can provide an important basis for targeting intervention and prevention activities for high-risk individuals.

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REFERENCES

1. Salazar, L. A., M. H. Hirata, N. Forti, J. Diament, S. D. Giannini, J. S. Issa, and R. D. C. Hirata. 2000. Puv II intron 15 polymorphism at the LDL receptor gene is associated with differences in serum lipid concentrations in subjects with low and high risk for coronary artery disease from Brazil. *Clin. Chim. Acta.* **293:** 75–88.
2. van Bockxmeer, F. M., Q. Liu, C. Mamotte, V. Burke, and R. Taylor. 2001. Lipoprotein lipase D9N, N291S and S447X polymorphisms: their influence on premature coronary heart disease and plasma lipids. *Atherosclerosis.* **157:** 123–129.
3. Ellsworth, D. L., P. Sholinsky, C. Jaquish, R. R. Fabsitz, and T. A. Manolio. 1999. Coronary heart disease at the interface of molecular genetic and preventive medicine. *Am. J. Prev. Med.* **16:** 122–133.
4. Brown, M. S., and J. L. Goldstein. 1997. The SREBP pathway: regulation of cholesterol metabolism by proteolysis of a membrane-bound transcription factor. *Cell.* **89:** 331–340.
5. Latasa, M. J., Y. S. Moon, K. H. Kim, and H. S. Sul. 2000. Nutritional regulation of the fatty acid synthase promoter in vivo: sterol regulatory element binding protein functions through an upstream region containing a sterol regulatory element. *Proc. Natl. Acad. Sci. USA.* **97:** 10619–10624.
6. Athanikar, J. N., and T. F. Osborne. 1998. Specificity in cholesterol regulation of gene expression by coevolution of sterol regulatory DNA element and its binding protein. *Proc. Natl. Acad. Sci. U.S.A.* **95:** 4935–4940.
7. Yokoyama, C., X. Wang, M. R. Briggs, A. Admon, J. Wu, X. Hua, and J. L. Goldstein. 1993. SREBP-1, a basic-helix-loop-helix-leucine zipper protein that controls transcription of the low density lipoprotein receptor gene. *Cell.* **75:** 187–197.
8. Hua, X., C. Yokoyama, J. Wu, M. R. Briggs, M. S. Brown, J. L. Goldstein, and X. Wang. 1993. SREBP-2, a second basic-helix-loop-helix-leucine zipper protein that stimulates transcription by binding to a sterol regulatory element. *Proc. Natl. Acad. Sci. U.S.A.* **90:** 11603–11607.
9. Iddon, C. R., J. Wilkinson, A. J. Bennett, J. Bennett, A. M. Salter, and J. A. Higgins. 2001. A role for smooth endoplasmic reticulum membrane cholesterol ester in determining the intracellular location and regulation of sterol-regulatory-element-binding protein-2. *Biochem. J.* **358:** 415–422.
10. Sakai, J., E. A. Duncan, R. B. Rawson, X. X. Hua, R. S. Brown, and J. L. Goldstein. 1996. Sterol-regulated release of SREBP-2 from cell membranes requires two sequential cleavage, one within transmembrane segment. *Cell.* **85:** 1037–1046.
11. Sakai, J., R. B. Rawson, P. J. Espenshade, D. Cheng, A. C. Seegmiller, J. L. Goldstein, and M. S. Brown. 1998. Molecular identification of the sterol-regulated luminal protease that cleaves SREBPs and controls lipid composition of animal cells. *Mol. Cell.* **2:** 505–514.
12. Miserez, A. R., P. Y. Muller, L. Barella, S. Barella, H. B. Staehelin, E. Leitersdorf, J. D. Kark, and Y. Friedlander. 2002. Sterol-regulatory

element-binding protein (SREBP)-2 contributes to polygenic hypercholesterolemia. *Atherosclerosis.* **164:** 15–26.
13. Shimano, H., I. Shimomura, R. E. Hammer, J. Herz, J. L. Goldstein, M. S. Brown, and J. D. Horton. 1997. Elevated levels of SREBP-2 and cholesterol synthesis in livers of mice homozygous for a targeted disruption of the SREBP-1 gene. *J. Clin. Invest.* **100:** 2115–2124.
14. Sakakura, Y., H. Shimano, H. Sone, A. Takahashi, K. Inoue, H. Toyoshima, S. Suzuki, and N. Yamada. 2001. Sterol regulatory element-binding proteins induce an entire pathway of cholesterol synthesis. *Biochem. Biophys. Res. Commun.* **286:** 176–183.
15. Shimano, H, N. Yahagi, M. Amemiya-Kudo, A. H. Hasty, J. Osuga, Y. Tamura, F. Shionoiri, Y. Iizuka, K. Ohashi, K. Harada, T. Gotoda, S. Ishibashi, and N. Yamada. 1999. Sterol regulatory element-binding protein-1 as a key transcription factor for nutritional induction of lipogenic enzyme genes. *J. Biol. Chem.* **274:** 415–422.
16. Horton, J. D., and I. Shimomura. 1999. Sterol regulatory element-binding proteins: activators of cholesterol and fatty acid biosynthesis. *Curr. Opin. Lipidol.* **10:** 143–150.
17. Vallett, S. M., H. B. Sanchez, J. M. Rosenfeld, T. F. Osborne. 1996. A direct role for sterol regulatory element binding protein in activation of 3-judroxy-3-methyglutaryl coenzyme A reductase gene. *J. Biol. Chem.* **271:** 12,247–12,253.
18. Sheng, Z., H. Otani, M. S. Brown, and J. L. Goldstein. 1995. Independent regulation of sterol regulatory element-binding proteins 1 and 2 in hamster liver. *Proc. Natl. Acad. Sci. U.S.A.* **92:** 935–938.
19. Foretz, M., C. Guichard, P. Ferre, and F. Foufelle. 1999. Sterol regulatory element binding protein-1c is a major mediator of insulin action on the hepatic expression of glucokinase and lipogenesis-related genes. *Proc. Natl. Acad. Sci. U.S.A.* **96:** 12,737–12,742.
20. Magana, M. M., S. H. Koo, H. C. Towle, and T. F. Osborne. 2000. Different sterol regulatory element-binding protein-1 isoforms utilize distinct co-regulatory factors to activate the promoter for fatty acid synthase. *J. Biol. Chem.* **275:** 4726–4733.
21. Shimomura, I., H. Shimano, J. D. Horton, J. L. Goldstein, and M. S. Brown. 1997. Differential expression of exons 1a and 1c in mRNAs for sterol regulatory element binding protein-1 in human and mouse organs and cultured cells. *J. Clin. Invest.* **99:** 838–845.
22. Misezez, A. R., G. Q. Cao, L. C. Probst, and H. H. Hobbs. 1997. Structure of the human gene encoding sterol regulatory element binding protein-2 (SREBP-2). *Genomics.* **40:** 31–40.
23. Hua, X., J. Wu, J. L. Goldstein, M. S. Brown, and H. H. Hobbs. 1995. Structure of the human gene encoding sterol regulatory element binding protein-1 (SREBF1) and localization of SREBF1 and SREBF2 to chromosomes 17p11.2 and 22q13. *Genomics.* **25:** 667–673.
24. Robinet, P., B. Vedie, G. Chironic, J. Gariepy, A. Aimon, N. Moatti, and J.-L. Paul. 2003. Characterization of polymorphic structure of SREBP-2 gene: role in atherosclerosis. *Atherosclerosis.* **168:** 381–387.
25. Muller, P. Y., and A. R. Miserez. 2002. Identification of mutations in the gene encoding sterol regulatory element binding protein (SREBP)-2 in hypercholesterolaemic subjects. *J. Med. Genet.* **39:** 271–275.
26. Xiao, Y., Z. Zhang, J. Wang, W. Zhu, Y. Zhao, S. Yan, and L. Yong. 2003. Effects of dietary intervention on hyperlipidemia in eight communities of Beijing, China. *Biomedical and Environmental Science.* **16:** 112–118.
27. Li, J. Z. Recommended methods for clinical laboratory measurement of serum total cholesterol. 1995. *Chinese Journal of Medical Laboratory Science.* **18:** 249–251 (in Chinese).
28. Vedie, B., X. Jeunemaitre, J. L. Megnien, V. Atger, A. Simon, and N. Moatti. 2001. A new DNA polymorphism in the 5′ untranslated region of the human SREBP-1a is related to development of atherosclerosis in high cardiovascular risk population. *Atherosclerosis.* **154:** 589–597.