Genetic Variation in the *OX40L/OX40* System and Plasma Lipid and Lipoprotein Levels in a Chinese Hypertriglyceridemic Population

Rui Liu,¹ Yan Qiao,¹ Yu Liu,² Xian Li,¹ Yucheng Chen,³ Ou Qiang,¹ and Huai Bai⁴

Aims: To investigate the variations of *OX40* (tumor necrosis factor receptor superfamily, member 4) and its ligand *OX40L* genes and their relationships with serum lipids and apolipoproteins (apo) levels in Chinese healthy individuals and patients with endogenous hypertriglyceridemia (HTG) in the Chengdu area. **Methods:** The genotypes and allele frequencies of the rs3850641 and rs17568 polymorphisms in the *OX40L* and *OX40* genes were assayed by polymerase chain reaction and restriction fragment length polymorphism. **Results:** In the case-control study, which included 126 HTG subjects and 206 normal control subjects, the frequencies of the G allele at the rs3850641 site and the G allele at the rs17568 site in the patients were similar to those observed in the controls. In the HTG group, subjects with G allele carriers of the rs3850641 site had lower serum high-density lipoprotein cholesterol and apo AI levels as compared to those of genotype AA. In the case group, subjects with G allele carriers of the rs17568 site had higher serum low-density lipoprotein cholesterol (LDL-C) levels, while controls had lower serum total serum cholesterol and LDL-C levels. **Conclusion:** These results suggest that the rs3850641 and rs17568 polymorphisms in the *OX40L* and *OX40* genes are associated with some of the lipid and lipoprotein variations in subjects with endogenous HTG and/or in the general population of Han Chinese.

Introduction

MANY EPIDEMIOLOGICAL STUDIES have shown that there is an inverse relationship between the plasma high-density lipoprotein (HDL)-cholesterol (HDL-C) level and the risk of premature atherosclerotic coronary heart disease (Boden, 2000). The genetic components determining the HDL levels are not fully elucidated. Previous studies suggested that altered plasma HDL-C levels have mainly been associated with the gene mutations or polymorphisms such as those in the cholesteryl ester transfer protein (*CETP*) gene (Boekholdt and Thompson, 2003), the apolipoprotein (apo) AI gene (the major apolipoprotein of HDL particles) (Dastani *et al.*, 2006), the lecithin:cholesterol acyl transferase (*LCAT*) gene (Kuivenhoven *et al.*, 1997a), the lipoprotein lipase (*LPL*), and hepatic lipase (*HL*) genes (Kuivenhoven *et al.*, 1997b; Cohen *et al.*, 1999), and the ATP-binding cassette A1 gene (*ABCA1*) (Alrasadi *et al.*, 2006). Recent study suggested that other genes such as the *OX40L/OX40* system showed a relationship with the HDL levels in addition to their inde-

pendent relationship to the CHD risk in mouse and human (Wang *et al.*, 2005).

The *OX40* ligand (*OX40L*, CD134L, and TNFSF4) is a member of the TNF superfamily that is expressed on activated B cells and dendritic cells (Godfrey *et al.*, 1994; Ohshima *et al.*, 1997). *OX40L* interacts with its receptor *OX40* (CD134), which is expressed on activated T cells (Al-Shamkhani *et al.*, 1997). *OX40L/OX40* is considered to act as a costimulator of T cells (Stuber and Strober, 1996; Flynn *et al.*, 1998), and has the potential to enhance inflammatory response in atherosclerosis plaques, that could be related to the immunoregulatory role of this pathway.

In a mouse model, Wang *et al.* (2005) found that *OX40L* is the gene underlying the AS-susceptibility locus 1 earlier identified by Paigen *et al.* (1987). In human subjects, the two case-control studies showed that genetic variations in *OX40L* are associated with MI and severity of CHD, and a haplotype (110NN) of the *OX40L* SNPs had a significantly higher plasma HDL-C levels than those carrying other haplotypes (Wang *et al.*, 2005). These authors further found that the genetic

¹Division of Peptides Related to Human Disease, West China Hospital, Sichuan University, Chengdu, Sichuan, People's Republic of China.

²Department of Biochemistry and Molecular Biology, West China School of Preclinical and Forensic Medicine, Sichuan University, Chengdu, Sichuan, People's Republic of China.

³Department of Cardiology, West China Hospital, Sichuan University, Chengdu, Sichuan, People's Republic of China.

⁴Laboratory of Genetic Disease and Perinatal Medicine, West China Second University Hospital, Sichuan University, Chengdu, Sichuan, People's Republic of China.

variation in other parts of the *OX40L*/*OX40* signaling pathway, such as rs17568 of the *OX40* gene, is also implicated in MI, and the variant of *OX40* gene such as rs17568 affects HDL levels in the population (Ria *et al.*, 2006). In the MDR model, there is an interaction between two SNPs in *OX40L* (rs3850641 and rs10912564) and one SNP in *OX40* (rs17568) in the disease phenotype (Wang *et al.*, 2005).

Up to now, there are few studies concerning the effects of the *OX40L* and *OX40* variants on intermediate phenotypes (such as lipid and lipoprotein levels) in other populations, besides a few studies on lipid levels in the Western populations (Wang *et al.*, 2005; Ria *et al.*, 2006). Therefore, it is important to investigate whether there is any effect of the polymorphisms on lipid profiles in the Chinese population, which is the largest population group in the world with different genetic background, diet, lifestyle, and environment. Moreover, due to the fact that patients with hypertriglyceridemia (HTG) were characterized by elevated serum triglyceride and reduced HDL-C levels, it is a matter of significance to investigate whether the *OX40L* and *OX40* polymorphisms influence serum lipid and apolipoprotein levels in the population.

The aim of the present study was to detect the *OX40L* and *OX40* gene polymorphisms with the relation to lipid profiles in the Chinese population. In addition, we also aimed to observe the genetic effect of the genes on the lipid levels of HTG subjects, who showed elevated triglycerides (TG) and low levels of HDL-C.

Materials and Methods

Subjects

For this study, blood samples were taken from 206 volunteers (127 men and 79 women, aged 51 ± 11 years) who were taking part in a routine health examination at three hospitals of the Sichuan University and the Sichuan Normal University in Chengdu, China. All these subjects were current or retired staff members of the Universities, and apparently healthy and unrelated individuals with serum TG < 1.82 mM (TG < 160 mg/dL) and total serum cholesterol (TC) < 6.21 mM (TC < 240 mg/dL). After a 12- to 14-h overnight fast, the blood from each individual was collected and analyzed for serum concentrations of lipids, lipoproteins, and apolipoproteins. For the case-control study, 126 HTG subjects (88 men and 38 women, aged 52 ± 15 years), consisting of serum TG ≥ 2.26 mM (TG ≥ 200 mg/dL) and TC < 6.21 mM (TC < 240 mg/dL) subjects were used as patients in the case-control study. The internal implications, such as CHD, diabetes mellitus, and hypertension, in the case group were also excluded. The informed consent was obtained, and the study was approved by the appropriate Institutional Review Board.

Quantitative analysis

TC and TG were measured by an enzymatic method (kits; Zhong Sen Co.). HDL-C was determined after sodium phosphotungstate/magnesium chloride precipitation of low-density lipoprotein by polyvinyl sulfate. Serum apo A-I, apo A-II, apo B100, apo C-II, apo C-III and apo E were quantified by a radial immunodiffusion kit developed by our laboratory (Liu, 1995). Low-density lipoprotein-cholesterol (LDL-C) was calculated using the Friedewald formula.

DNA extraction and genotyping

Genomic DNA was isolated from 500 μ L peripheral blood according to the method of Erlich (1989). The polymerase chain reactions (PCRs) were performed in a final volume of 25 μ L containing 10% 10 $\times$ PCR buffer, 2 mM MgCl₂, 0.2 mM dNTPs, 0.6 U Taq DNA polymerase (MBI Fermentas), and sense and antisense primers, 0.5 μ M of each. We used 100 ng DNA per PCR reaction. The investigated DNA sequences were amplified by the following primers: 5'-CAC ACA TTG CTC CGC TAT TAT T-3' as a sense primer (P1), and 5'-AGT CAC TGA TAT ACC TGG TCT ACC AA-3' as an antisense primer (P2) for *Mun* I (Fermentas Biolabs; rs3850641 site); 5'-CCA GCC ACG CAG CCC CAG AA-3' as a sense primer (P3) and 5'-CTG GGT GGG GTC CAC AGG AGG G-3' as an antisense primer (P4) for *Mbo* II (Fermentas Biolabs; rs17568 site). These primers yielded a PCR product of 188 base pairs (bp) and 213 bp spanning the *Mun* I and *Mbo* II polymorphic sites, respectively. Amplifications were carried out in the MyCycler™ thermal cycler system (Bio-Rad) under the following conditions: introductory denaturation at 94°C for 5 min, then 30 amplification cycles, denaturation at 94°C for 30 s, primers binding (annealing) at 55°C (rs3850641 site) or 67°C (rs17568 site) for 30 s, and chain elongation at 72°C for 30 s. PCR ended with 7-min chain elongation at 72°C. Amplification products were digested with the restrictive enzyme *Mun* I or *Mbo* II (MBI Fermentas). For *Mun* I site, in the case of wild type (genotype AA), the PCR product was digested into DNA fragments of 160 and 28 bp. The mutant G allele did not undergo digestion with the enzyme *Mun* I. For *Mbo* II site, in the case of mutant genotype (genotype GG), the PCR product was digested into DNA fragments of 183 and 30 bp. The wild-type A allele did not undergo digestion with the enzyme *Mbo* II. DNA fragments obtained after respective restrictive enzyme digestion and the DNA size marker were electrophoresed on a 10% polyacrylamide gel and stained with ethidium bromide. For result documentation, gel pictures were taken under ultraviolet light. DNA sequencing was used to confirm results on PCR-restriction fragment length polymorphism genotyping assays.

Statistical analysis

Allele frequencies of the *OX40L*/*OX40* polymorphisms were estimated by gene counting. The Hardy-Weinberg equilibrium was tested in cases and controls by a chi-square test. The allele and genotype frequencies were compared between cases and controls by chi-square analysis. To evaluate the effect of the *OX40*/*OX40L* polymorphisms on the variation of quantitative variables of lipid and apolipoprotein, an ANOVA was carried out. The relation between the rs3850641 and rs17568 alleles in the *OX40L* and *OX40* genes and the dependent variables (lipid and apolipoprotein) was studied using correlation analysis and partial correlations analysis (adjusting for covariables: age, gender, and body-mass index). Statistical analyses were performed using SPSS statistical software. Significance was assumed for $p < 0.05$.

Results

Baseline characteristics of the participants in the population

It shows a typical characteristics of type IV hyperlipoproteinemia (endogenous HTG) in lipid and apolipoprotein profile in a case group when compared with normal controls (Table 1).

TABLE 1. CLINICAL AND BIOCHEMICAL CHARACTERISTICS OF CONTROL AND HYPERTRIGLYCERIDEMIA GROUPS

	Control group (n=206)	HTG group (n=126)
Age (years)	50.6±11.0	51.7±15.1
Gender (M/F)	127/79	88/38
BMI (kg/m ²)	23.0±3.2	25.0±2.7 ^a
TG (mM)	1.19±0.37	3.46±1.54 ^a
TC (mM)	5.02±0.67	5.12±0.75
HDL-C (M)	1.39±0.49	0.98±0.26 ^a
LDL-C (M)	3.11±0.67	2.70±0.80 ^a
apoA-I (mg/dL)	131±23	115±22 ^a
apoA-II (mg/dL)	27.94±4.26	28.04±4.84
apoB100 mg/dL)	81.89±56.43	93.15±15.90 ^a
apoC-II (mg/dL)	4.59±1.66	8.51±3.54 ^a
apoC-III (mg/dL)	11.23±2.97	19.32±7.42 ^a
apoE (mg/dL)	4.26±1.11	6.08±2.22 ^a

Compared with control group, ^a*p*<0.01.

HDL-C, high-density lipoprotein-cholesterol; LDL-C, low-density lipoprotein-cholesterol; HTG, hypertriglyceridemia; TC, total serum cholesterol; TG, triglycerides.

In case group, the serum TG level is increased and HDL-C decreased, and apo B100, apo C-II, apoC-III, and apo E increased, and apo A-I and LDL-C decreased (*p*<0.01, respectively).

OX40/OX40L allele frequencies

The PCR-amplified fragments (containing rs3850641 and rs17568 polymorphic sites of *OX40L* and *OX40* genes, respectively) from each sample was digested with the restriction enzyme *Mun*I for the rs3850641 site and *Mbo*II for the rs17568 site, and analyzed by polyacrylamide gel electrophoresis (Figs. 1 and 2).

FIG. 1. Polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP) analysis of rs3850641 site of the *OX40L* gene. The three patterns represent the A- and G-allele-containing genotypes, namely the homozygous AA and GG forms and the heterozygous AG form. Lane M shows the size markers. Lanes 1 and 4 show the AA genotype; 2 and 3, the AG genotype; and 5, the GG genotype.

FIG. 2. PCR-RFLP analysis of rs17568 site of the *OX40* gene. The three patterns represent the A- and G-allele-containing genotypes, namely the homozygous AA and GG forms and the heterozygous AG form. Lane M shows the size markers. Lanes 1 and 2 show the AG genotype; 3 and 5, the AA genotype; and 4, the GG genotype.

Genotypes of the rs3850641 and rs17568 polymorphisms were found to be in the Hardy-Weinberg equilibrium in both the HTG and control groups, respectively. The frequency data are presented in Table 2. The A and G allele frequencies of the *OX40L* gene at rs3850641 in HTG and normal control groups were 0.833, 0.167, and 0.845, 0.155, respectively; the A and G allele frequencies of the *OX40* gene at rs17568 in HTG and the control groups were 0.694, 0.306 and 0.731, 0.269, respectively. Both G allele frequencies of the two polymorphisms in HTG subjects were not different from those in the normal controls, respectively (*p*>0.05).

In addition, the frequency of the G allele at rs3850641 site of the *OX40L* gene was not significantly different from that

TABLE 2. GENOTYPE AND ALLELE FREQUENCY FOR THE rs17568 AND rs3850641 SITES OF THE *OX40/OX40L* GENES IN CONTROL AND HYPERTRIGLYCERIDEMIA GROUPS

	Frequency		
	Control (n=206)	HTG (n=126)	Total (n=332)
<i>OX40L</i> rs3850641			
Genotype			
AA	0.709 (146)	0.698 (88)	0.705 (234)
AG	0.272 (56)	0.270 (34)	0.271 (90)
GG	0.019 (4)	0.032 (4)	0.024 (8)
Allele			
A	0.845 (348)	0.833 (210)	0.840 (558)
G	0.155 (64)	0.167 (42)	0.160 (106)
<i>OX40</i> rs17568			
Genotype			
AA	0.524 (108)	0.460 (58)	0.500 (166)
AG	0.413 (85)	0.468 (59)	0.434 (144)
GG	0.063 (13)	0.071 (9)	0.066 (22)
Allele			
A	0.731 (301)	0.694 (175)	0.717 (476)
G	0.269 (111)	0.306 (77)	0.283 (188)

The distribution of the rs17568 and rs3850641 polymorphisms agrees with the Hardy-Weinberg equilibrium in both the control and HTG groups.

TABLE 3. MEAN VALUES ($\bar{x} \pm s$) OF SERUM LIPID AND APOLIPOPROTEIN LEVELS FOR rs3850641 SITE OF THE OX40L GENE IN THE CONTROL AND HYPERTRIGLYCERIDEMIA GROUPS

	Control		HTG	
	AA (n=146)	AG+GG (n=64)	AA (n=88)	AG+GG (n=38)
TG (mM) ^a	1.19±0.38	1.13±0.36	3.54±1.71	3.23±0.99
TC (mM)	5.03±0.66	4.96±0.69	5.18±0.73	4.96±0.77
HDL-C (mM)	1.34±0.35	1.51±0.72	1.00±0.28	0.89±0.24 ^b
LDL-C (mM)	3.13±0.65	2.92±0.97	2.53±0.92	2.57±0.75
apoA-I (mg/dL)	130±22.81	132.14±22.75	117.49±22.50	108.91±18.41 ^b
apoA-II(mg/dL)	28.32±6.20	27.94±4.70	28.53±5.37	26.77±3.11
apoB100 (mg/dL)	79.15±13.55	88.23±13.25	94.36±15.63	90.25±16.13
apoC-II(mg/dL)	4.73±1.68	4.23±1.55	8.69±3.17	8.08±7.93
apoC-III (mg/dL)	11.31±3.03	11.01±2.85	19.25±3.77	17.83±2.89
apoE (mg/dL)	4.36±1.13	4.03±1.02	6.27±2.35	5.65±1.82
BMI(kg/m ²)	22.97±3.04	23.09±3.57	25.06±2.98	24.94±2.15

The relationships between the genotype and plasma HDL-C levels remained significant even with adjustment for age, gender, and BMI, whereas other values were similar after these adjustments.

^aLog-transformed TG values were used in the analysis.

Compared with genotype AA carriers in the same group, ^b $p < 0.05$.

reported in a Swedish population (0.155 vs. 0.121, $p > 0.05$) (Wang *et al.*, 2005) and U.S. population (0.155 vs. 0.16, $p > 0.05$) (Mälarstig *et al.*, 2008). There is no significant difference of the frequency of the G allele at rs17568 site of the OX40 gene between Chinese (0.269) and either Swedish (0.23) (Ria *et al.*, 2006) or Japanese (0.25) (Mashimo *et al.*, 2008). These results suggested that racial difference among these populations was not evident at the rs3850641 and rs17568 sites of the genes.

Effects of polymorphic sites in the OX40/OX40L genes on serum concentration of lipids and apolipoproteins

Because of the relative small number of subjects with the GG (both rs3850641 and rs17568) genotype, heterozygotes and homozygotes for the G allele were pooled in the analysis.

To assess the possible impact of polymorphic sites in the OX40/OX40L genes on lipid metabolism, we analyzed the serum lipids and lipoprotein levels in different genotypes of

the rs3850641 and rs17568 polymorphisms in both control and patient groups (Tables 3–4).

In the HTG group, subjects with G allele carriers of the rs3850641 site of the OX40L gene had a lower serum mean concentration of HDL-C and apo AI as compared to those of genotype AA (HDL-C: 0.89±0.24 mM vs. 1.00±0.28 mM, $p < 0.05$; apo AI: 108.91±18.41 mg/dL vs. 117.49±22.50 mg/dL, $p < 0.05$) (Table 3, Figure 3A, B). When we calculated the Pearson correlation coefficient, the G allele was significantly associated with lower HDL-C and apoA-I levels in univariate ($p = 0.027$ and 0.041, respectively) and multivariable ($p = 0.035$ and 0.044, respectively) analysis that adjusted for age, gender, and body-mass index in the group. In the case group, subjects who were G allele carriers of the rs17568 site of the OX40 gene had a higher serum mean concentration of LDL-C, while controls had lower serum TC and LDL-C levels (Table 4, Figure 4A–C). When we calculated the Pearson correlation coefficient, the G allele in the HTG group was significantly

TABLE 4. MEAN VALUES ($\bar{x} \pm s$) OF SERUM LIPID AND APOLIPOPROTEIN LEVELS FOR rs17568 SITE OF THE OX40 GENE IN CONTROL AND HYPERTRIGLYCERIDEMIA GROUPS

	Control		HTG	
	AA (n=108)	AG+GG (n=98)	AA (n=58)	AG+GG (n=68)
TG (mM) ^a	1.21±0.35	1.14±0.39	3.60±1.52	3.32±1.53
TC (mM)	5.10±0.63	4.93±0.71 ^b	4.93±0.85	5.26±0.66
HDL-C (mM)	1.35±0.38	1.43±0.58	0.95±0.27	0.97±0.28
LDL-C (mM)	3.18±0.63	2.96±0.86 ^b	2.31±0.84	2.74±0.86 ^b
apoA-I (mg/dL)	130.63±20.74	131.64±24.72	114.11±22.16	115.00±21.52
apoA-II(mg/dL)	27.70±4.22	27.17±4.21	27.38±4.67	28.42±5.01
apoB100 (mg/dL)	78.20±10.85	77.60±15.92	93.90±17.63	92.21±15.06
apoC-II(mg/dL)	4.60±1.59	4.58±1.72	8.31±3.87	8.71±3.19
apoC-III (mg/dL)	11.42±2.84	11.05±3.09	19.42±8.74	19.19±6.00
apoE (mg/dL)	4.23±0.89	4.29±1.29	6.23±2.39	5.95±2.11
BMI(kg/m ²)	22.93±2.79	23.05±3.63	24.50±2.64	25.28±2.81

The relationships between the genotype and plasma TC and LDL-C levels remained significant even with adjustment for age, gender, and BMI, whereas other values were similar after these adjustments.

^aLog-transformed TG values were used in the analysis.

Compared with genotype AA carriers in the same group, ^b $p < 0.05$.

FIG. 3. High-density lipoprotein-cholesterol (HDL-C) and apoA-I levels by rs3850641 of the *OX40L* polymorphism ratios (allele carrier status). A significant decrease in the HDL-C (**A**) and apoA-I (**B**) levels in the hypertriglyceridemia group was identified in G-allele carriers ($n=38$) versus AA genotype carriers ($n=88$). The graphs plot individual data points with mean values representing mean and standard deviation ($p=0.035$ and 0.044 , respectively, by multivariable analysis). Overlapping data points were not plotted.

associated with higher LDL-C levels in univariate ($p=0.039$) and multivariable ($p=0.042$) analysis that adjusted for age, gender, and body-mass index, while controls were significantly associated with lower TC and LDL-C levels in univariate ($p=0.011$ and 0.004 , respectively) and multivariable ($p=0.008$ and 0.024 , respectively) analysis that adjusted for age, gender and body-mass index.

Discussion

The human *OX40L* and *OX40* genes appear to be polymorphic in the Western populations (Wang *et al.*, 2005; Mälarstig *et al.*, 2008). The present study shows that the allele frequencies of the *OX40L* rs3850641 SNP and the *OX40* rs17568 SNP in the Chinese population (Han Chinese), which is the largest population group in the world, are also polymorphic, and in addition, racial differences in the distribution of alleles at the rs3850641 and rs17568 sites of the genes were not evident.

This study first detected the association of the *OX40L* and *OX40* polymorphisms with an endogenous hypertriglyceridemia risk in a population. Our work does not provide evidence in favor of *OX40L* rs3850641 and *OX40* rs17568 being candidate genes for conferring genetic susceptibility to HTG in a South-West Chinese population.

Previous studies (Wang *et al.*, 2005) showed that *Tnfrsf4*^{-/-} mice fed either chow or a high-fat diet had higher levels of plasma total cholesterol and HDL-C than did controls, and human subjects with a haplotype (110NN) of the *OX40L* SNPs had significantly higher plasma HDL-C levels than those carrying other haplotypes. In a recent study, Ria *et al.* (2006) in human subjects demonstrated that G allele carriers of rs17568 of the *OX40* gene displayed a lower HDL-C concentration in a Swedish MI case-control study. Our patients with HTG featured decreased HDL-C levels with higher serum TG showed a lower serum mean concentration of HDL-C and apo AI, the major component of HDL-C, in G allele carriers as compared to those of genotype AA at the rs3850641 site in the *OX40L* gene, suggesting that this polymorphism is associated with HDL-C and apo AI levels in HTG subjects. In addition, the patients with G allele carriers had a higher serum mean concentration of LDL-C compared to those of genotype AA at the rs17568 site in the *OX40* gene. Our study extended the previous findings that genetic components of the *OX40L/OX40* system were associated with some of the lipid and/or lipoprotein concentrations in HTG individuals in addition to their suggested relation with HDL-C levels and CHD risk in Western populations. Whether these polymorphisms contribute to lipid alterations associated with other metabolic disorders,

FIG. 4. Total serum cholesterol (TC) and low-density lipoprotein-cholesterol (LDL-C) levels by rs17568 of *OX40* polymorphism ratios (allele carrier status). A significant increase in the LDL-C (**A**) was identified in G-allele carriers ($n=68$) versus AA genotype carriers ($n=58$) in case group, while significant decrease in the TC (**B**) and LDL-C (**C**) levels in control group. The graphs plot individual data points with mean values representing mean and standard deviation. ($p=0.042$, 0.008 and 0.024 , respectively, by multivariable analysis). Overlapping data points were not plotted.

and thus potential CHD risk in our population, awaits additional studies.

The control samples of the present study offered the opportunity of studying genetic determinants of lipid levels in Asian Chinese population-based samples, not selected in terms of HTG. The control subjects showed a significantly lower serum TC and LDL-C levels in G allele carriers, of the rs17568 site of the *OX40* gene compared with AA homozygotes. We did not find that the rs3850641 site of the *OX40L* gene contributes to significant variations of lipid levels in the control population at large. As far as we know, this is the first

study of the two gene variants on lipid levels in an oriental general population, and this result suggested that the polymorphisms of the *OX40* gene, rather than the *OX40L* gene, might play a role in determining some of the lipid levels in normal Han Chinese.

Although up to now the exact relationship between the *OX40L* and *OX40* system and CHD risk or intermediate phenotypes such as lipid levels was not well defined, the data currently available have indicated that *OX40L* and *OX40* might be related to some lipid components (TC, HDL-C, and LDL-C), which link to the risk of disease (CHD).

Conclusion

The present study provides evidence that rs3850641 and rs17568 polymorphisms in the *OX40L* and *OX40* genes are associated with some of the lipid and lipoprotein variations in subjects with endogenous HTG and/or the general population in Han Chinese. However, these polymorphisms are not associated with the risk of HTG in the population. Studies in an expanded sample will draw further conclusions.

Acknowledgments

This work was supported by a grant from the National Natural Sciences Foundation of China (No.39770322), and Research Seed Fund from West China Second Hospital of Sichuan University (to HB). The authors thank patients with HTG and controls who donated blood samples for this study.

Author Disclosure Statement

No competing financial interests exist.

References

- Al-Shamkhani A, Mallett S, Brown MH, *et al.* (1997) Affinity and kinetics of the interaction between soluble trimeric OX40 ligand, a member of the tumor necrosis factor superfamily, and its receptor OX40 on activated T cells. *J Biol Chem* 272:5275–5282.
- Alrasadi K, Ruel IL, Marcil M, *et al.* (2006) Functional mutations of the ABCA1 gene in subjects of French-Canadian descent with HDL deficiency. *Atherosclerosis* 188:281–291.
- Bingwen L (1995) Enzymatic method and radial immunodiffusion measurement and its application in clinical aspects. In: Keqing W (ed), *Lipoprotein and Atherosclerosis*, 1st edition. People's Health Publish House, Beijing, pp 359–360.
- Boden WE (2000) High-density lipoprotein cholesterol as an independent risk factor in cardiovascular disease: assessing the data from Framingham to the Veterans Affairs High-Density Lipoprotein Intervention Trial. *Am J Cardiol* 86:19L–22L.
- Boekholdt SM, Thompson JF (2003) Natural variation as a tool in understanding the role of CETP in lipid levels and disease. *J Lipid Res* 44:1080–1093.
- Cohen JC, Vega GL, Grundy SM (1999) Hepatic lipase: new insights from genetic and metabolic studies. *Curr Opin Lipidol* 10:259–267.
- Dastani Z, Dangoisse C, Boucher B, *et al.* (2006) A novel non-sense apolipoprotein A-I mutation (apoA-I (E136×)) causes low HDL cholesterol in French Canadians. *Atherosclerosis* 185:127–136.
- Erlach HA (1989) PCR technology: principles and applications for DNA amplification, 1st edition. Stockton Press, New York.
- Flynn S, Toellner KM, Raykundalia C, *et al.* (1998) CD4 T cell cytokine differentiation: the B cell activation molecule, OX40 ligand, instructs CD4 T cells to express interleukin 4 and up-regulates expression of the chemokine receptor, Bir-1. *J Exp Med* 188:297–304.
- Godfrey WR, Fagnoni FF, Harara MA, *et al.* (1994) Identification of a human OX-40 ligand, a costimulator of CD4+ T cells with homology to tumor necrosis factor. *J Exp Med* 180:757–762.
- Kuivenhoven JA, Pritchard H, Hill J, *et al.* (1997a) The molecular pathology of lecithin:cholesterol acyltransferase (LCAT) deficiency syndromes. *J Lipid Res* 38:191–205.
- Kuivenhoven JA, Groenemeyer BE, Boer JM, *et al.* (1997b) Ser447stop mutation in lipoprotein lipase is associated with elevated HDL cholesterol levels in normolipidemic males. *Arterioscler Thromb Vasc Biol* 17:595–599.
- Mälärstig A, Eriksson P, Rose L, *et al.* (2008) Genetic variants of tumor necrosis factor superfamily, member 4 (TNFSF4), and risk of incident atherothrombosis and venous thromboembolism. *Clin Chem* 54:833–840.
- Mashimo Y, Suzuki Y, Hatori K, *et al.* (2008) Association of TNFRSF4 gene polymorphisms with essential hypertension. *J Hypertens* 26:902–913.
- Ohshima Y, Tanaka Y, Tozawa H, *et al.* (1997) Expression and function of OX40 ligand on human dendritic cells. *J Immunol* 159:3838–3848.
- Paigen B, Mitchell D, Reue K, *et al.* (1987) Ath-1, a gene determining atherosclerosis susceptibility and high density lipoprotein levels in mice. *Proc Natl Acad Sci U S A* 84:3763–3767.
- Ria M, Eriksson P, Boquist S, *et al.* (2006) Human genetic evidence that OX40 is implicated in myocardial infarction. *Biochem Biophys Res Commun* 339:1001–1006.
- Stuber E, Strober W (1996) The T cell-B cell interaction via OX40-OX40L is necessary for the T cell-dependent humoral immune response. *J Exp Med* 183:979–989.
- Wang X, Ria M, Kelmenson PM, *et al.* (2005) Positional identification of TNFSF4, encoding OX40 ligand, as a gene that influences atherosclerosis susceptibility. *Nat Genet* 37:365–372.

Address correspondence to:

Huai Bai, MD, PhD

Laboratory of Genetic Disease and Perinatal Medicine

West China Second University Hospital

Sichuan University

Chengdu 610041

Sichuan

People's Republic of China

E-mail: baihuai60@yahoo.com.cn