Effects of genetic variants on lipid parameters and dyslipidemia in a Chinese population[S]

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Abstract A number of recent genome-wide association (GWA) studies have identified several novel genetic determinants of plasma lipid and lipoprotein concentrations in European populations. However, it is still unclear whether these loci identified in Caucasian GWA studies also exert the same effect on lipid and lipoprotein concentrations in a Chinese population. We genotyped 10 single-nucleotide polymorphisms (SNPs) in nine loci in a Chinese Han population sample (n = 4,192) and assessed the associations of these SNPs with metabolic traits, using linear regression adjusted for age, gender, diabetes status, and body mass index. Three variants (rs12654264, $P \sim 1.7 \times 10^{-6}$; rs3764261, $P \sim 7.1 \times 10^{-7}$; and rs4420638, $P \sim 1.1 \times 10^{-3}$) showed strong evidence for association with total cholesterol; four variants (rs780094, $P \sim 1.8 \times 10^{-11}$; rs17145738, $P \sim 5.0 \times 10^{-7}$; rs326, $P \sim 2.3 \times 10^{-6}$; and rs439401, $P \sim 2.2 \times 10^{-5}$) showed strong evidence for association with triglycerides, four variants (rs17145738, $P \sim 1.9 \times 10^{-4}$; rs326, $P \sim 9.7 \times 10^{-4}$; rs1800588, $P \sim 1.5 \times 10^{-7}$; and rs3764261, $P \sim 4.3 \times 10^{-14}$) showed strong evidence for association with HDL-cholesterol (HDL-C), two variants (rs12654264, $P \sim 2.3 \times 10^{-5}$; and rs4420638, $P \sim 3.6 \times 10^{-4}$) showed strong evidence for association with LDL-C, and four variants (rs326, $P \sim 2.8 \times 10^{-3}$; rs1800588, $P \sim 6.1 \times 10^{-4}$; rs3764261, $P \sim 2.0 \times 10^{-3}$; and rs4420638, $P \sim 9.4 \times 10^{-5}$) showed strong evidence for association with total cholesterol-HDL-C-related ratio. These SNPs generated strong combined effects on lipid traits and dyslipidemia. Our findings indicate that the variants that associated with metabolic traits in Europeans may also play a role in a Chinese Han population.—Liu, Y., D. Zhou, Z. Zhang, Y. Song, D. Zhang, T. Zhao, Z. Chen, Y. Sun, D. Zhang, Y. Yang, Q. Xing, X. Zhao, H. Xu, and L. He. **Effects of genetic variants on lipid parameters and dyslipidemia in a Chinese population. *J. Lipid Res.* 2011.** 52: **354–360.**

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Dyslipidemia is a common health problem in developing countries, including China (1). A vast line of evidence has demonstrated that plasma lipids and lipoprotein concentrations are important risk factors for atherosclerosis and related vascular diseases, which are the leading causes of death in China and the rest of the world (2, 3). Although plasma lipid concentrations are strongly influenced by smoking, diet, level of physical activity, and other lifestyles choices, twin and family studies suggest that about 50% of the variation in HDL cholesterol (HDL-C), LDL-C, and total cholesterol (TC) levels is genetically determined (4).

Since 2008, genome-wide association (GWA) studies of plasma lipid levels have further identified several common variants associated with plasma lipid levels, exerting a modest fraction of variance (2% or less) (5–15). Some newly identified genes are potential new drug targets, so these recent genetic advances have broadened our understanding of basic metabolic pathways and can improve patient classification, disease diagnosis, and treatment strategies (14). However, because of the known differences in genome-wide linkage disequilibrium patterns among

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Abbreviations: BMI, body mass index; CHD, coronary heart disease; CI, confidence interval; CVD, cardiovascular disease; GWA, genome-wide association; HDL-C, HDL cholesterol; LDL-C, LDL cholesterol; MAF, minor allele frequency; OR, odds ratio; SNP, single-nucleotide polymorphism; TC, total cholesterol; TG, triglyceride.

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[S] The online version of this article (available at http://www.jlr.org) contains supplementary data in the form of four tables.

different ethnic groups, it is still unclear whether the loci identified in European GWA studies also exert their effects on lipid concentrations in Chinese. Accordingly, using a sample consisting of 4,192 individuals of Chinese Han origin, we aimed to determine whether those common variants in nine loci were associated with blood lipid and lipoprotein concentrations. In addition, many studies show that HDL-C-related ratios (e.g., TC/HDL-C, LDL-C/HDL-C, and non-HDL-C) are powerful predictors of coronary heart disease (CHD) risk, and some investigators propose that these "cholesterol ratios" are simple approaches to lipid risk assessment (16–18). Thus, we also investigated whether these polymorphisms also show associations with lipid indexes (TC/HDL-C, LDL-C/HDL-C, and non-HDL-C) and whether these genetic loci exert combined effects on these lipid parameters and dyslipidemia.

MATERIALS AND METHODS

Study design

We selected 10 loci from recent GWA studies that have been reported to be associated with lipid levels. We evaluated the effect of the 10 single-nucleotide polymorphisms (SNPs) on lipid levels in a Chinese sample population of 4,192 individuals, in what was designed to be a case-control study (19) for type 2 diabetes (2,041 non-type 2 diabetes controls, 239 patients with impaired glucose tolerance and/or impaired fasting glucose, and 1,912 type 2 diabetes patients). We then constructed a genotype score and further investigated the cumulative effect of allelic dosage of risk alleles on dyslipidemia.

Participants

From March to October 2006, a total of 4,192 40- to 80-year-old Han Chinese subjects (including 1,503 men and 2,689 women) were recruited from Shanghai. Subjects were eligible for enrollment if *1*) they were stable residents for at least 20 years in the area; *2*) they were free of severe psychological disorders, physical disabilities, and cancer and had no history of stroke, CHD, Alzheimer's disease, or dementia; *3*) and they had not been currently diagnosed with tuberculosis, AIDS, and other communicable diseases. Their diabetes status was defined in accordance with World Health Organization criteria. Dyslipidemia was diagnosed according to criteria set forth by the National Cholesterol Education Program-Adult Treatment Panel III and divided into four phenotypes (18): *1*) patients with isolated hypertriglyceridemia: serum triglycerides (TG) $\geq$ 1.7 mmol/l, or taking medication, and TC < 6.2 mmol/l; *2*) patients with isolated hypercholesterolemia: TC $\geq$ 6.2 mmol/l, or taking medication, and TG < 1.7 mmol/l; *3*) patients with mixed hyperlipidemia: TG $\geq$ 1.7 mmol/l, and TC $\geq$ 6.2 mmol/l; and *4*) patients with isolated low HDL-C: HDL-C $\leq$ 1.03 mmol/l for males and $\leq$1.29 mmol/l for females, without hypertriglyceridemia or hypercholesterolemia. Detailed information for subgroups is summarized in supplementary Tables I and II.

Home interviews were conducted by trained physicians or public health workers from the Pudong and Baoshan Centers for Disease Control and Prevention and community hospitals in Shanghai, China. For all individuals, height, weight, hip and waist circumference, and blood pressure were measured by trained medical professionals using a standardized protocol. Body mass index (BMI) was calculated as weight (kg)/[height (m)]^2. Obese subjects were defined as those with a BMI of 27.5kg/m^2 or greater, according to the recommendation for Asians (20). Total cholesterol, HDL-C, LDL-C, TG, hemoglobin A1c, and fasting plasma glucose levels were measured enzymatically according to standard methods with a modular P800 model autoanalyzer (Roche, Mannheim, Germany) with reagents (Roche Diagnostics GmbH, Mannheim, Germany). Non-HDL-C was calculated by subtracting HDL-C from TC.

In the present study, a standard informed consent to undergo the protocol, which was reviewed and approved by the ethics committee of the Shanghai Institute for Biological Sciences, was given by all participants after the nature of the study had been fully explained to them.

Candidate variants selection

Previous studies (6–8, 11), especially GWA studies, have identified a large number of loci exhibiting compelling evidence for associations between common variants and lipoprotein or lipid concentrations. Fifteen genes and loci (*MLXIPL*, *GCKR*, *APOE*, *PCSK9*, *CETP*, *GALNT2*, *CILP2*, *LPL*, *APOB*, *LIPC*, *LDLR*, *ABCA1*, *ANGPTL3*, *APOA1*, and *HMGCR*) were considered for this replication study in a Chinese population. Some of these susceptible SNPs have a very low minor allele frequency (MAF) in Chinese according to HapMap data. To ensure that our experiment had enough statistical power, we selected only those SNPs with an MAF higher than 10%. In total, 10 representative SNPs in or near nine loci identified from previous studies (5–8, 11) were included in the present study, as follows: rs3764261in the cholesteryl ester transfer protein (*CETP*) gene; rs12654264 in the 3-hydroxy-3-methylglutaryl-CoA reductase (*HMGCR*) gene; rs780094 in the glucokinase regulatory protein (*GCKR*) gene; rs4846914 in the polypeptide *N*-acetyl-galactosaminyltransferase 2 (*GALNT2*) gene; rs17145738 near the MLX interacting protein-like (*MLXIPL*) gene; rs1529729 in the LDL receptor (*LDLR*) gene; rs326 in the lipoprotein lipase (*LPL*) gene; rs1800588 in the hepatic lipase (*LIPC*) gene; and rs4420638 and rs439401 (r^2 = 0.05) in the apolipoprotein E (*APOE*) gene cluster.

Genotyping

High-molecular-weight genomic DNA was prepared from venous blood, using a QuickGene 610L model automatic DNA/RNA extraction system (Fujifilm, Tokyo, Japan). All representative SNP genotyping experiments were done using TaqMan technology on an ABI7900 system (Applied Biosystems, Foster City, CA). Standard 5-μl PCR reactions were carried out using TaqMan Universal PCR Master Mix reagent kits according to the manufacturer's guidelines. Genotype data were obtained from about 97.5% of the DNA samples, and replicate quality control samples (5% samples) were included and genotyped with 100% concordance.

Statistical analysis

SHEsis software was used to perform the Hardy-Weinberg equilibrium test (21). For metabolic traits, continuous data are presented as means ± SD or median (interquartile range) values. Plasma TG levels and TC/HDL-C and LDL-C/HDL-C ratios were logarithmically transformed due to skewed distributions. To control these confounding factors, we used gender, age, BMI, and diabetes status as covariates in the multivariable linear regression analysis. Bonferroni correction was used to control type I error, according to 10 SNPs investigated for every lipid phenotypic trait; a *P* value $\leq$0.005 was considered significant. To establish the closest best-fit model for lipid-associated SNPs, we carried out a logistic regression analysis by comparing additive, dominant, and recessive models with age and gender as covariates. In the additive model, homozygotes for the minor allele (R/R) and heterozygotes

TABLE 1. Association of SNPs with TC, TG, HDL-C, and LDL-C

SNP	Chr	Gene	Minor allele	MAF (%)[a]	R/R[b]	C/R[b]	C/C[b]	Effect[c]	P_{add}[d]	P_{dom}[d]	P_{rec}[d]
TC											
rs4846914	1	*GALNT2*	A	21	4.47 ± 0.91	4.51 ± 0.89	4.48 ± 0.91	0.01	0.70	0.76	0.56
rs780094	2	*GCKR*	C	47.7	4.42 ± 0.88	4.51 ± 0.91	4.51 ± 0.92	−0.05	0.02	5.5×10^{-3}	0.21
rs12654264	5	*HMGCR*	A	48.3	4.39 ± 0.84	4.49 ± 0.92	4.59 ± 0.94	−0.09	1.7×10^{-6}	1.0×10^{-4}	7.7×10^{-5}
rs17145738	7	*MLXIPL*	T	10.5	4.58 ± 1.19	4.50 ± 0.90	4.49 ± 0.90	0.03	0.32	0.24	0.43
rs326	8	*LPL*	G	17.6	4.49 ± 0.97	4.49 ± 0.89	4.49 ± 0.91	0.01	0.80	0.76	0.86
rs1800588	15	*LIPC*	T	36.8	4.51 ± 0.99	4.51 ± 0.88	4.46 ± 0.91	0.04	0.05	0.20	0.06
rs3764261	16	*CETP*	A	14.7	4.61 ± 0.96	4.63 ± 0.92	4.46 ± 0.90	0.14	7.1×10^{-7}	0.09	3.7×10^{-7}
rs1529729	19	*LDLR*	C	23	4.48 ± 0.85	4.53 ± 0.97	4.47 ± 0.88	0.04	0.11	0.90	0.04
rs439401	19	*APOE* cluster	C	40.1	4.50 ± 0.93	4.48 ± 0.92	4.50 ± 0.88	−0.01	0.69	0.49	0.87
rs4420638	19	*APOE* cluster	C	11.6	4.71 ± 1.02	4.56 ± 0.93	4.47 ± 0.88	0.1	1.1×10^{-3}	3.3×10^{-3}	0.02
TG											
rs4846914	1	*GALNT2*	A	21	1.55(1.05-2.15)	1.41(1.01-2.01)	1.44(1.03-2.13)	0.08	0.1	0.06	0.87
rs780094	2	*GCKR*	C	47.7	1.34(0.97-1.87)	1.43(1.01-1.41)	1.53(1.11-2.27)	−0.16	1.8×10^{-11}	3.5×10^{-8}	7.5×10^{-8}
rs12654264	5	*HMGCR*	A	48.3	1.46(0.99-1.38)	1.43(1.01-2.11)	1.43(1.04-2.12)	0.02	0.54	0.48	0.73
rs17145738	7	*MLXIPL*	T	10.5	1.21(0.86-2.06)	1.39(0.97-1.90)	1.45(1.04-2.15)	−0.17	5.0×10^{-7}	1.3×10^{-6}	0.02
rs326	8	*LPL*	G	17.6	1.29(0.99-1.98)	1.36(0.94-1.98)	1.47(1.05-2.14)	−0.13	2.3×10^{-6}	1.1×10^{-5}	3.9×10^{-3}
rs1800588	15	*LIPC*	T	36.8	1.48(1.06-2.21)	1.43(1.03-2.11)	1.42(1.00-2.06)	0.01	0.1	0.13	0.28
rs3764261	16	*CETP*	A	14.7	1.51(1.10-2.18)	1.41(1.03-2.08)	1.44(1.02-2.10)	0.05	0.25	0.32	0.36
rs1529729	19	*LDLR*	C	23	1.34(0.96-2.03)	1.42(1.02-2.11)	1.45(1.03-2.09)	−0.01	0.55	0.53	0.82
rs439401	19	*APOE* cluster	C	40.1	1.49(1.08-2.29)	1.46(1.04-2.11)	1.38(0.99-2.02)	0.1	2.2×10^{-5}	1.5×10^{-3}	2.8×10^{-4}
rs4420638	19	*APOE* cluster	C	11.6	1.41(0.99-2.33)	1.48(1.04-2.13)	1.41(1.01-2.03)	0.11	0.01	0.87	7.1×10^{-3}
HDL-C											
rs4846914	1	*GALNT2*	A	21	1.20 ± 0.30	1.22 ± 0.31	1.21 ± 0.31	−0.01	0.38	0.98	0.33
rs780094	2	*GCKR*	C	47.7	1.22 ± 0.33	1.23 ± 0.31	1.19 ± 0.30	0.01	0.24	0.80	0.14
rs12654264	5	*HMGCR*	A	48.3	1.21 ± 0.31	1.21 ± 0.31	1.23 ± 0.32	−0.01	0.25	0.77	0.15
rs17145738	7	*MLXIPL*	T	10.5	1.28 ± 0.34	1.24 ± 0.34	1.21 ± 0.30	0.04	1.9×10^{-4}	0.15	2.5×10^{-4}
rs326	8	*LPL*	G	17.6	1.24 ± 0.31	1.24 ± 0.31	1.20 ± 0.31	0.03	9.7×10^{-4}	0.15	1.0×10^{-3}
rs1800588	15	*LIPC*	T	36.8	1.25 ± 0.32	1.22 ± 0.32	1.19 ± 0.30	0.04	1.5×10^{-7}	2.0×10^{-4}	2.6×10^{-6}
rs3764261	16	*CETP*	A	14.7	1.38 ± 0.36	1.27 ± 0.33	1.19 ± 0.30	0.07	4.3×10^{-14}	1.3×10^{-7}	1.1×10^{-11}
rs1529729	19	*LDLR*	C	23	1.24 ± 0.32	1.22 ± 0.32	1.20 ± 0.31	0.01	0.18	0.67	0.15
rs439401	19	*APOE* cluster	C	40.1	1.23 ± 0.32	1.21 ± 0.32	1.21 ± 0.32	0.01	0.51	0.40	0.79
rs4420638	19	*APOE* cluster	C	11.6	1.20 ± 0.28	1.21 ± 0.31	1.22 ± 0.32	−0.02	0.08	0.08	0.79
LDL-C											
rs4846914	1	*GALNT2*	A	21	2.80 ± 0.77	2.84 ± 0.75	2.78 ± 0.74	0.02	0.20	0.80	0.10
rs780094	2	*GCKR*	C	47.7	2.79 ± 0.74	2.81 ± 0.74	2.80 ± 0.76	−0.01	0.53	0.41	0.82
rs12654264	5	*HMGCR*	A	48.3	2.72 ± 0.72	2.80 ± 0.74	2.86 ± 0.76	−0.07	2.3×10^{-5}	7.1×10^{-5}	2.7×10^{-3}
rs17145738	7	*MLXIPL*	T	10.5	2.74 ± 0.93	2.83 ± 0.74	2.79 ± 0.74	−0.04	0.12	0.74	0.08
rs326	8	*LPL*	G	17.6	2.90 ± 0.78	2.81 ± 0.75	2.79 ± 0.74	0.02	0.20	0.38	0.25
rs1800588	15	*LIPC*	T	36.8	2.78 ± 0.76	2.80 ± 0.73	2.81 ± 0.73	−0.01	0.45	0.88	0.35
rs3764261	16	*CETP*	A	14.7	2.76 ± 0.74	2.84 ± 0.78	2.79 ± 0.73	−0.05	0.04	0.90	0.02
rs1529729	19	*LDLR*	C	23	2.75 ± 0.75	2.82 ± 0.78	2.80 ± 0.72	−0.02	0.34	0.49	0.14
rs439401	19	*APOE* cluster	C	40.1	2.80 ± 0.78	2.76 ± 0.74	2.85 ± 0.73	−0.04	0.02	1.7×10^{-3}	0.66
rs4420638	19	*APOE* cluster	C	11.6	2.99 ± 0.85	2.85 ± 0.77	2.77 ± 0.74	0.09	3.6×10^{-4}	1.4×10^{-3}	9.1×10^{-3}

[a] MAF, minor allele frequency; Chr, chromosome.
[b] R/R, homozygous for minor allele; C/R, heterozygous for minor allele; C/C, homozygous for common allele. Data are shown as means ± SD (e.g., for TC SNPs) or as medians (25%–75% range) (e.g., for TG SNPs).
[c] Effects are measured as additive effects, which correspond to the average change in phenotype when the major allele is replaced by the minor allele.
[d] *P* values were calculated with adjustment for age, sex, BMI, and diabetes status. P_{add}, value under the additive model; P_{dom}, value under the dominant model; P_{rec}, value under the recessive model.

(R/C) and homozygotes for the major allele (C/C) were coded to an ordered categorical variable for the genotype (2, 1, and 0, respectively). The dominant model was defined as R/R plus R/C versus C/C, and the recessive model was defined as R/R versus R/C plus C/C. The model that gave the lowest Akaike information criterion value was considered the best fitting model for the respective SNP.

On the basis of results for the genotype-phenotype association analyses, we performed cumulative analysis with the lipid traits that had at least four associated SNPs. We assumed that an individual SNP would have a similarly modest effect on lipid traits and dyslipidemia and then constructed a genotype score on the basis of the number of risk alleles that were carried by each subject. The cumulative effects of four SNPs for TC/HDL-C, four SNPs for plasma TG, four SNPs for HDL-C, and seven SNPs for dyslipidemia were assessed by multivariable linear or logistic regression, using the categories of risk allele carried as an independent variable. Statistical analyses were performed using SPSS software (SPSS Inc., Chicago, IL).

RESULTS

In our study, 10 representative SNPs in or near nine loci were genotyped, and none of the 10 SNPs showed statistical deviation from Hardy-Weinberg equilibrium ($P > 0.05$). For the 10 SNPs, the success rates were 99.1% (rs4846914), 99.2% (rs780094), 98.2% (rs12654264), 99.5% (rs17145738), 98.6% (rs326), 98.7% (rs1800588), 92.7% (rs3764261), 99.2% (rs1529729), 91.2% (rs439401), and 96.7% (rs4420638). Analysis of the missing data showed no significant differences between cases and controls.

SNPs associated with TC, HDL-C, LDL-C, and TG

Associations among each of the 10 SNPs with levels of TC, TG, HDL-C, and LDL-C found by multiple linear regression analyses after adjustment for age, gender, BMI, and diabetes status are shown in **Table 1**. Three variants showed strong evidence of association with total cholesterol: rs12654264 near the *HMGCR* gene (TC increased at 0.09 mmol/l per T allele); rs3764261 in the *CETP* gene (0.14 mmol/l per A allele); and rs4420638 in the *APOE* gene cluster (0.10 mmol/l per C allele). Four variants showed strong evidence of association with TG after multiple testing corrections, namely, rs780094 in the *GCKR* gene (TG concentration increase of 0.16 mmol/l per T allele); rs17145738 in the *MLXIPL* gene (0.17 mmol/l per C allele); rs326 in the *LPL* gene (0.13 mmol/l per A allele); and rs439401 in the *APOE* gene cluster (0.10 mmol/l per C allele). Four variants showed strong evidence of association with HDL-C, namely, rs17145738 in the *MLXIPL* gene (HDL-C concentration increase of 0.04 mmol/l per T allele); rs326 in the *LPL* gene (0.03 mmol/l per G allele); rs1800588 in the *LIPC* gene (0.04 mmol/l per T allele); and rs3764261 in the *CETP* gene (0.07 mmol/l per A allele). Two variant, rs12654264 in the *HMGCR* gene (0.07 mmol/l per T allele) and rs4420638 in the *APOE* gene cluster (0.09 mmol/l per C allele), showed strong evidence of association with LDL-C after multiple testing corrections. The additive model gave the lowest Akaike information criterion value and was therefore considered the best-fit genetic model for each variant, which was consistent with previous GWA studies (5, 7, 8, 11, 12, 15, 22).

SNPs associated with TC/HDL-C, LDL-C/HDL-C, non-HDL-C, and dyslipidemia

As shown in **Table 2**, rs326 in the *LPL* gene ($P \sim 2.8 \times 10^{-3}$); rs1800588 in the *LIPC* gene ($P \sim 6.1 \times 10^{-4}$); rs3764261 in the *CETP* gene ($P \sim 2.0 \times 10^{-3}$); and rs4420638 in the APOE gene cluster ($P \sim 9.4 \times 10^{-5}$) were associated with TC/HDL-C. Variant associations with LDL-C/HDL-C, which included rs12654264 in the *HMGCR* gene, rs3764261 in the *CETP* gene, and rs4420638 in the *APOE* gene cluster, achieved significance. For non-HDL-C, significant associations were detected in three polymorphisms (rs780094 in the *GCKR* gene, rs12654264 in the *HMGCR* gene, and rs4420638in the *APOE* gene cluster).

Allele and genotype distributions of the variants in dyslipidemia and nondyslipidemia are summarized in **Table 3**. After we adjusted for age, gender, BMI, and diabetes status, six loci showed associations with dyslipidemia, as follows: rs780094 in the *GCKR* gene ($P \sim 0.02$); rs17145738 in the *MLXIPL* gene ($P \sim 1.1 \times 10^{-3}$); rs326 in the *LPL* gene ($P \sim 0.01$); rs1800588 in the *LIPC* gene ($P \sim 9.8 \times 10^{-3}$); rs3764261 in the *CETP* gene ($P \sim 4.6 \times 10^{-4}$); and rs4420638 in the *APOE* gene cluster ($P \sim 0.05$).

Combined effects of genetic variants on lipid levels and dyslipidemia

In the present study, three lipid level indexes (TC/HDL-C, TG, and HDL-C) showed associations with four susceptible SNPs. Because a person may carry 0, 1, or 2 risk alleles for

TABLE 2. Association of SNPs with TC/HDL-C, LDL-C/HDL-C, and non-HDL-C

					TC/HDL-C		LDL-C/HDL-C		non-HDL-C	
SNP	Chr	Gene	Minor allele	MAF (%)	Effect[a]	P^b	Effect[a]	P^b	Effect[a]	P^b
rs4846914	1	*GALNT2*	A	21	−0.016	0.57	0.006	0.76	0.004	0.86
rs780094	2	*GCKR*	C	47.7	−0.054	0.02	−0.010	0.56	−0.052	5.0×10^{-3}
rs12654264	5	*HMGCR*	A	48.3	−0.057	0.01	−0.047	6.8×10^{-3}	−0.086	3.3×10^{-6}
rs17145738	7	*MLXIPL*	T	10.5	−0.081	0.02	−0.023	0.43	−0.011	0.73
rs326	8	*LPL*	G	17.6	−0.084	2.8×10^{-3}	−0.029	0.20	−0.021	0.39
rs1800588	15	*LIPC*	T	36.8	−0.072	6.1×10^{-4}	−0.045	0.01	0.004	0.85
rs3764261	16	*CETP*	A	14.7	−0.088	2.0×10^{-3}	−0.090	3.1×10^{-4}	0.066	0.01
rs1529729	19	*LDLR*	C	23	0.003	0.94	−0.007	0.74	0.028	0.21
rs439401	19	*APOE* cluster	C	40.1	−0.019	0.31	−0.042	0.02	−0.013	0.51
rs4420638	19	*APOE* cluster	C	11.6	0.134	9.4×10^{-5}	0.119	1.4×10^{-5}	0.116	7.1×10^{-5}

[a]Effects were measured as additive effects, which corresponds to the average change in phenotype when the major allele is replaced by the minor allele.

[b]*P* values were calculated using the additive model and adjusted for age, sex, BMI, and diabetes status.

each SNP, the potential number of risk alleles at four loci for each subject ranged from 0 to 8. Because only a small number of subjects had three or fewer risk alleles in analysis, these groups were combined into one group for data display and analysis.

As shown in **Table 4**, the TC–HDL-C ratio increased from 3.57 for those with three or fewer risk alleles to 4.14 for those carrying all eight risk alleles (P for trend, ~9.2×10^{-11}). TG levels increased from 1.16 mmol/l for those with three or fewer risk alleles to 1.68 mmol/l for those carrying all eight risk alleles (P for trend, ~6.4×10^{-23}). HDL-C levels decreased from 1.38 mmol/l for those carrying three or fewer risk alleles to 1.14 mmol/l for those carrying all eight risk alleles (P for trend, ~4.1×10^{-23}). For the dyslipidemia risk score, we included seven SNPs (rs780094 in the *GCKR* gene; rs12654264 in the *HMGCR* gene; rs17145738 in the *MLXIPL* gene; rs326 in the *LPL* gene; rs1800588 in the *LIPC* gene; rs3764261 in the *CETP* gene; and rs4420638 in the *APOE* gene cluster) that showed associations with at least one lipid trait. For the same reason, subjects with 5 or fewer risk alleles and subjects with 12 or more risk alleles were divided into two groups. The proportion of individuals with dyslipidemia rising with increasing genotype score is shown in supplementary Table III (odds ratio [OR], ~1.14; 95% confidence interval [CI], 1.09–1.20; P for trend, ~1.1×10^{-8}).

DISCUSSION

In this study, of the 10 lipid-related SNPs identified in European populations, we confirmed that 8 SNPs (at seven genetic loci) were associated with lipid parameters or dyslipidemia in a Chinese population. We observed several loci influenced more than one lipid trait (Table 1 and supplementary Table IV). To determine whether associations were dependent on each other, we performed multiple linear regression analyses of SNPs by including age, gender, BMI, diabetes status, and other lipid traits as covariates. Considering the tight relationship among HDL-C, LDL-C, and other cholesterol indexes (including TC, TC/HDL-C, LDL-C/HDL-C, and non-HDL-C), we focused only on the overlapping associations among TGs, HDL-C, and LDL-C. Results showed that the significant associations of *MLXIPL* and *LPL* with one lipid trait were retained when adjustments were made by including the other lipid trait as covariates, which suggested that rs17145738 in the *MLXIPL* gene and rs326 in the *LPL* gene were independently associated with TG and HDL-C. Given the biological relationships of the lipid traits, the independent associations between a locus and two lipid traits confirmed each other and reinforced the evidence that the locus is involved in lipid metabolism. We need more detailed studies to elucidate *MLXIPL* and *LPL* roles in lipid metabolism.

Combination analysis showed cumulative genetic effects on lipid parameters (Table 3) and a substantially increased risk in dyslipidemia. However, previous GWA studies (7, 11, 12, 14) in Europeans and our study in Chinese showed that each variant conferred a modest effect and that the

TABLE 3. Association of candidate SNPs in dyslipidemia and non-dyslipidemia individuals

SNPs	Chr	Gene	Minor allele	Group(s)	Allelic distribution n(%)		Genotype distribution n(%)			P^b	OR	95% CI
					R[a]	C[a]	R/R[a]	C/R[a]	C/C[a]			
rs4846914	1	*GALNT2*	A	Dyslipidemia	1086(20.8)	4142(79.2)	119(4.6)	848(32.4)	1647(63)			
				Nondyslipidemia	657(21.3)	2421(78.7)	71(4.6)	515(33.5)	953(61.9)	0.49	0.96	(0.86–1.08)
rs780094	2	*GCKR*	C	Dyslipidemia	2455(46.8)	2787(53.2)	583(22.2)	1289(49.2)	749(28.6)			
				Nondyslipidemia	1518(49.4)	1554(50.6)	368(24)	782(50.9)	386(25.1)	0.02	0.90	(0.82–0.98)
rs12654264	5	*HMGCR*	A	Dyslipidemia	2503(48.3)	2679(51.7)	630(24.3)	1243(48)	718(27.7)			
				Nondyslipidemia	1467(48.1)	1585(51.9)	366(24)	735(48.2)	425(27.9)	0.98	1.00	(0.91–1.10)
rs17145738	7	*MLXIPL*	T	Dyslipidemia	511(9.7)	4739(90.3)	19(0.7)	473(18)	2133(81.3)			
				Nondyslipidemia	367(11.9)	2725(88.1)	23(1.5)	321(20.8)	1202(77.7)	1.1×10^{-3}	0.78	(0.67–0.91)
rs326	8	*LPL*	G	Dyslipidemia	871(16.7)	4349(83.3)	76(2.9)	719(27.5)	1815(69.5)			
				Nondyslipidemia	584(19.2)	2464(80.8)	58(3.8)	468(30.7)	998(65.5)	0.01	0.86	(0.76–0.96)
rs1800588	15	*LIPC*	T	Dyslipidemia	1859(35.6)	3357(64.4)	335(12.8)	1189(45.6)	1084(41.6)			
				Nondyslipidemia	1187(38.8)	1875(61.2)	227(14.8)	733(47.9)	571(37.3)	9.8×10^{-3}	0.88	(0.80–0.97)
rs3764261	16	*CETP*	A	Dyslipidemia	676(13.7)	4256(86.3)	53(2.1)	570(23.1)	1843(74.7)			
				Nondyslipidemia	471(16.6)	2373(83.4)	42(3)	387(27.2)	993(69.8)	4.6×10^{-4}	0.79	(0.69–0.90)
rs1529729	19	*LDLR*	C	Dyslipidemia	1165(22.2)	4077(77.8)	141(5.4)	883(33.7)	1597(60.9)			
				Nondyslipidemia	749(24.3)	2329(75.7)	86(5.6)	577(37.5)	876(56.9)	0.07	0.90	(0.81–1.01)
rs439401	19	*APOE* cluster	C	Dyslipidemia	1965(40.6)	2873(59.4)	389(16.1)	1187(49.1)	843(34.8)			
				Nondyslipidemia	1092(39.1)	1704(60.9)	209(14.9)	674(48.2)	515(36.8)	0.11	1.08	(0.98–1.20)
rs4420638	19	*APOE* cluster	C	Dyslipidemia	616(12.1)	4480(87.9)	39(1.5)	538(21.1)	1971(77.4)			
				Nondyslipidemia	325(10.8)	2689(89.2)	23(1.5)	279(18.5)	1205(80)	0.05	1.15	(0.99–1.33)

Abbreviations: Chr = chromosome.
[a] R/R, homozygous for minor allele; C/R, heterozygous for minor allele; C/C, homozygous for common allele.
[b] P values were calculated under the additive model and adjusted for age, sex, BMI and diabetes status.

TABLE 4. Cumulative effects of the risk factors on TC–HDL-C, TGs and HDL-C

Variable	Genotype score[a]						Effect (95% CI)	*P*value[e]
TC/HDL-C								
Genotype score[b]	⩽3	4	5	6	7	8		
N	403	911	1349	882	163	12		
TC/HDL-C	3.57(2.97–4.26)	3.64(3.01–4.36)	3.80(3.14–4.48)	3.94(3.25–4.61)	4.05(3.31–4.67)	4.14(3.65–4.89)	0.09 (0.06∼1.12)	9.2×10^{-11}
TG								
Genotype score[c]	⩽3	4	5	6	7	8		
N	258	695	1138	1079	515	68		
Mean TG (mmol/l)	1.16(0.87–1.59)	1.3(0.91–1.83)	1.42(1.01–2.06)	1.54(1.08–2.19)	1.50(1.14–2.34)	1.68(1.19–2.68)	0.07 (0.06∼ 0.09)	6.4×10^{-23}
High TG (>2.25 mmol/l) n (%)	30 (11.6)	108 (15.5)	219 (19.2)	245 (22.8)	137 (26.6)	24 (35.3)		
HDL-C								
Genotype score[d]	⩽3	4	5	6	7	8		
N	31	144	564	1155	1327	587		
Mean HDL-C (mmol/l)	1.38 ± 0.41	1.30 ± 0.33	1.27 ± 0.33	1.24 ± 0.31	1.19 ± 0.30	1.14 ± 0.28	−0.04 (−0.05∼−0.04)	4.1×10^{-23}
Low HDL-C (<1.03mmol/l) N (%)	7 (22.6)	29 (20.1)	135 (23.9)	302 (26.1)	419 (31.6)	225 (38.3)		

[a]The genotype score represents the number of risk alleles associated with TC/HDL-C, TG, or HDL-C.
[b]Number of risk alleles at the four SNPs: *LPL* rs326, *LIPC* rs1800588, *CETP* rs3764261, *APOE* cluster rs4420638.
[c]Number of risk alleles at the four SNPs: *GCKR* rs780094, *MLXIPL* rs17145738, *LPL* rs326, *APOE* cluster rs439401.
[d]Number of risk alleles at the four SNPs: *MLXIPL* rs17145738, *LPL* rs326, *LIPC* rs1800588, *CETP* rs3764261.
[e]*P* values were calculated under the additive model and adjusted for age, sex, BMI, and diabetes status.

variants identified could explain only a small fraction (5% to 10% cumulatively) of interindividual variability in lipid or lipoprotein levels. Johansen et al. (23) found that rare variants incrementally increased the proportion of genetic variation contributing to hypertriglyceridemia. Our study and previous GWA studies were focused on common variants, which may have limited our findings. Future studies of rare variants, copy number variations, and other genetic structure variations would help us to delineate the genetic mechanism underlying lipid metabolism.

Plasma level measurements fluctuate with diet, exercise levels, and some random factors, so they could reflect only the condition of a specific time. However, plasma lipid level-associated DNA sequence variants may represent a lifelong impact on lipid levels, and therefore, they add predictive information beyond a single measurement of blood lipids. Blood lipid concentrations have a causal role in the development of cardiovascular disease (CVD). It has been estimated that a 1% decrease in serum LDL-C concentration reduces the risk of CVD by approximately 1%, and each 1% increase in HDL-C concentration reduces the risk of CVD by approximately 2% (24, 25). According to the study by Kathiresan et al. (6), SNPs associated with levels of either LDL-C or HDL-C were independently associated with a risk of first myocardial infarction, ischemic stroke, or death from CHD. Previous studies describe TC/HDL-C, LDL-C/HDL-C, and non-HDL-C, which reflect the proportion of atherogenic to antiatherogenic lipid fractions, as powerful predictors for CHD, myocardial infarction, and other vascular diseases (26). Our study found that several loci (the *APOE* cluster and the *LPL*, *LIPC*, *CETP*, *GCKR*, and *HMGCR* genes) were associated with these cholesterol ratios. In addition to standard clinical factors, the information provided by these variants can modestly improve reclassification of patients at clinical risk of developing CVD for individual subjects. In addition, some of these genes are currently being targeted for drug development and design. Genetic variation may have affects on the patient's response to drugs, although the drugs may show a modest effect on lipid levels. For instance, the *HMGCR* gene encodes the rate-limiting enzyme for cholesterol synthesis and is the drug target for statins, commonly used for treating high LDL values. Interindividual differences in response to statins are associated with a common alternatively spliced *HMGCR* variant (27, 28). Hence, one can see that these genetic studies have broadened our understanding of basic metabolic pathways and will improve classification, diagnosis, and treatment strategies.

There are several points worth noting. First, the sample in our study was enriched with type 2 diabetes cases, similar to several previous GWA studies in Europeans. Three reasons were considered for including type 2 diabetes subjects: *1*) to increase the statistical power of our study; *2*) previous studies suggested that effects on lipids seen in diabetic individuals are independent of the disease (12); and *3*) diabetic status was included as a covariate in the regression analysis to adjust the potential confounding effect.

Second, we assigned an equal weight to each risk allele in the cumulative studies, whereas if we had been able to

accurately estimate the exact contribution of each allele to levels of cholesterol, the results would have given a better reflection of the actual situation.

Third, there are considerable differences between Caucasian and Chinese populations. In our study, rs4846914 in the *GALNT2* gene and rs1529729 in the *LDLR* gene showed no associations with plasma lipid levels. The minor allele frequencies of rs4846914 and rs1529729 were lower in the Chinese population (0.21 and 0.23, respectively) than in European populations (0.40 and 0.44, respectively). In addition to the lower frequency of these SNPs, the differences in the linkage disequilibrium pattern may contribute to the lack of an association between these two loci and plasma lipid concentrations in the Chinese population. One limitation to this study was that only one tag SNP was included in each locus; so, to understand fully the genetic background of plasma lipid concentrations in the Chinese population, both GWA studies and deep resequencing of candidate genes are required in further research.

In summary, we successfully replicated a set of SNPs associated with baseline lipid levels in a Chinese Han population. Also, we further identified these variants that showed combined effects on lipid parameters and dyslipidemia.

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