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RESEARCH ARTICLE

Genetic variants influencing lipid levels and risk of dyslipidemia in Chinese population

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Abstract. Recently, several human genetic and genomewide association studies (GWAS) have discovered many genetic loci that are associated with the concentration of the blood lipids. To confirm the reported loci in Chinese population, we conducted a cross-section study to analyse the association of 25 reported SNPs, genotyped by the ABI SNaPshot method, with the blood levels of total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C) and triglycerides (TG) in 1900 individuals by multivariate analysis. Logistic regression was applied to assess the association of the genetic loci with the risk of different types of dyslipidemia. Our study has convincingly identified that 12 of 25 studied SNPs were strongly associated with one or more blood lipid parameters (TC, LDL, HDL and TG). Among the 12 associated SNPs, 10 significantly influence the risk of one or more types of dyslipidemia. We firstly found four SNPs (rs12654264 in *HMGCR*; rs2479409 in *PCSK9*; rs16996148 in *CILP2*, *PBX4*; rs4420638 in *APOE-C1-C4-C2*) robustly and independently associate with four types of dyslipidemia (MHL, mixed hyperlipidemia; IHTC, isolated hypercholesterolemia; ILH, isolated low HDL-C; IHTG, isolated hypertriglyceridemia). Our results suggest that genetic susceptibility is different on the same candidate locus for the different populations. Meanwhile, most of the reported genetic variants strongly influence one or more plasma lipid levels and the risk of dyslipidemia in Chinese population.

Keywords. dyslipidemia; lipid levels; single-nucleotide polymorphisms; cardiovascular disease; genetics.

Introduction

Cardiovascular disease (CVD) is the leading cause of death worldwide, and predicted to be the major cause of morbidity and mortality in most developing nations by 2020 globally (Celermajer *et al.* 2012). One of the major risk factors of CVD is dyslipidemia (Shanmugasundaram *et al.* 2010), a disorder of lipid and lipoprotein metabolism (Radovica *et al.* 2014). Dyslipidemia is characterized by the increased total cholesterol (TC), low-density lipoprotein-cholesterol (LDL-C), or triglyceride (TG) concentration, or the declined high-density lipoprotein-cholesterol (HDL-C) concentration in the

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blood (Dyslipidemia (http://www.merckmanuals.com/professional/endocrine_and_metabolic_disorders/lipid_disorders/dyslipidemia.html)). Causes that increase dyslipidemia are obesity and high fat intake; however, many individuals vary in their responses to dietary cholesterol, indicating the importance of genetic factors (Radovica *et al.* 2014).

Recent human genetic and genomewide association studies (GWAS) have discovered many genetic loci that are associated with the concentrations of different blood lipids (Abifadel *et al.* 2003; Kathiresan *et al.* 2008; Kooner *et al.* 2008; Wallace *et al.* 2008; Willer *et al.* 2008; Chasman *et al.* 2009; Deo *et al.* 2009; Teslovich *et al.* 2010; Kim *et al.* 2011; Sarzynski *et al.* 2011; Inouye *et al.* 2012). More than a hundred of them are at or near one of the following genes: *ABCA1*, *APOB*, *APOE-C1-C4-C2*, *BCL7B*, *TBL2*, *MLXIPL*, *CELSR2*, *PSRC1*, *SORT1*, *GALNT2*, *GCKR*, *HMGCR*, *LIPG*, *ACAA2*, *PCSK9*, *MLXIPL*, *LPL*, *TRIR1*, *LIPC*, *CETP*, *CILP2*, *PBX4*, *APOA1-C3-A4-A5*, *ZNF259*, *BUD13* (Abifadel *et al.* 2003; Kathiresan *et al.* 2008; Kooner *et al.* 2008; Wallace *et al.* 2008; Willer *et al.* 2008; Chasman *et al.* 2009; Deo *et al.* 2009; Teslovich *et al.* 2010; Kim *et al.* 2011; Sarzynski *et al.* 2011; Inouye *et al.* 2012). These genetic variants are reported to be significantly influence one or more lipids in the blood. However, most of these associations were not confirmed in Chinese population, especially relationship between these genetic variants and the risk of dyslipidemia remains unclear. Here, we report the associations of the most-informative SNPs from previous studies with four blood lipid parameters: TC, HDL-C, LDL-C and TG in Chinese population and to provide more information to characterize the genetic factors that influence the blood lipid levels. Currently, predicted dyslipidemia depends on the analysis of environmental risk factors. If we built a database of lipid susceptibility loci of Chinese population, we can predict dyslipidemia with the combination of genetic and environmental risk factors. It will certainly improve the ability to predict dyslipidemia.

Materials and methods

Subjects and data collection

This study was a cross-sectional study regarding chronic diseases and risk factors conducted in Chengdu in 2014. Two urban communities of health examination population in Chengdu were selected randomly. Individuals were enrolled by a random sampling design. Approval was obtained from the Institutional Review Boards of the Sichuan Academy of Medical Sciences and Sichuan Provincial People's Hospital. Written informed consent was obtained from all subjects prior to this study. This study was conducted in accordance with the tenets of the Declaration of Helsinki.

In the present study, a total number of 1900 participants, including 746 men and 1154 women, were recruited by the Hospital of the University of Electronic Science and Technology of China, Sichuan Provincial People's Hospital. Demographic features of the subjects are listed in table 1. All participants were Han Chinese from southern China. Dyslipidemia was diagnosed according to the criteria set by Joint Committee for Developing Chinese guidelines on Prevention Treatment of Dyslipidemia in Adults and classified into four phenotypes (Prevention *et al.* 2016). (i) Isolated hypertriglyceridemia (IHTG) was defined as having TG $\geq$ 1.7 mmol/L or on medication and TC $<$ 5.2 mmol/L; (ii) isolated hypercholesterolemia (IHTC) was defined as having TC $\geq$ 5.2 mmol/L or on medication and TG $<$ 1.7 mmol/L; (iii) mixed hyperlipidemia (MHL) was defined as having TG $\geq$ 1.7 mmol/L and TC $\geq$ 5.2 mmol/L; and (iv) isolated low HDL-C (ILH) was defined as having HDL-C $\leq$ 1.0 mmol/L. Normal control is defined as subjects without any dyslipidemia.

Hypertension was defined as SBP $\geq$ 140 mmHg, DBP $\geq$ 90 mmHg, or current medication for hypertension (James *et al.* 2014). Height, weight, blood pressure, TC, TG, LDL-C and HDL-C were measured. Height and weight were measured with the subject standing barefoot in light clothes. Body mass index (BMI) was calculated as weight (kg) divided by square of height (m). BMI was divided into three categories: low BMI $<$ 24 kg/m^2; middle BMI = 24$-$28 kg/m^2; high BMI $>$ 28 kg/m^2. High glucose was defined as $>$6.1 mmol/L. Blood pressure was measured by standard mercury sphygmomanometer on the right arm in sitting position after the participants have rested at least for 5 min. Blood samples were collected from all the participants after an overnight fasting. All the biochemical assessments were conducted in the clinical laboratory of Sichuan Provincial People's Hospital. Concentrations of fasting glucose (Glu), TC, HDL-C, TG, and LDL-C were measured using an auto analyzer (Hitachi 717, Hitachi Instruments, Tokyo, Japan).

SNP selection

Among the recent six GWAS and other five genetic studies on the plasma levels of different blood lipids, 18 genetic loci, including *ABCA1*, *APOB*, *APOE-C1-C4-C2*, *BCL7B-TBL2-MLXIPL*, *CELSR2*, *CELSR2-PSRC1-SORT1*, *GALNT2*, *GCKR*, *HMGCR*, *LIPG-ACAA2*, *PCSK9*, *MLXIPL*, *LPL*, *TRIR1*, *LIPC*, *CETP*, *CILP2-PBX4*, *APOA1-C3-A4-A5-ZNF259-BUD13* were associated with one or more lipid traits (Abifadel *et al.* 2003; Kathiresan *et al.* 2008; Kooner *et al.* 2008; Wallace *et al.* 2008; Willer *et al.* 2008; Chasman *et al.* 2009; Deo *et al.* 2009; Teslovich *et al.* 2010; Kim *et al.* 2011; Sarzynski *et al.* 2011; Inouye *et al.* 2012) (table 1 in electronic supplementary material at http://www.ias.ac.in/jgenet/). For HDL cholesterol, the

Table 1. Basic characteristic of the study subjects.

	Men ($n = 746$)	Women ($n = 1154$)
Age (years), mean ± SD*	60.06 ± 13.21	59.61 ± 10.85
BMI (kg/m^2), mean ± SD*	24.25 ± 3.08	23.84 ± 3.18
Glu (mmol/L), median (Q1, Q3)*	4.97 (4.6, 5.4)	4.85 (4.52, 5.36)
IHTG, *n* (%)	120 (16.1)	98 (8.5)
TC (mmol/L), mean ± SD	4.57 ± 0.029	4.67 ± 0.44
TG (mmol/L), median (Q1, Q3)*	2.28 (1.91, 2.96)	2.22 (1.93, 2.80)
HDL-C (mmol/L), mean ± SD*	1.04 ± 0.015	1.11 ± 0.23
LDL-C (mmol/L), mean ± SD*	2.77 ± 0.031	2.80 ± 0.44
IHTC, *n* (%)	174 (23.3)	405 (35.1)
TC (mmol/L), mean ± SD	5.92 ± 0.373	5.91 ± 0.56
TG (mmol/L), median (Q1, Q3)*	1.11 (0.86, 1.39)	1.11 (0.89, 1.38)
HDL-C (mmol/L), mean ± SD*	1.49 ± 0.018	1.53 ± 0.30
LDL-C (mmol/L), mean ± SD*	3.70 ± 0.046	3.61± 0.72
MLP, *n* (%)	130 (17.4)	200 (17.3)
TC (mmol/L), mean ± SD	5.98 ± 0.042	6.19 ± 0.78
TG (mmol/L), median (Q1, Q3)*	2.38 (1.93, 3.53)	2.25 (1.92, 2.97)
HDL-C (mmol/L), mean ± SD*	1.18 ± 0.013	1.26 ± 0.24
LDL-C (mmol/L), mean ± SD*	3.86 ± 0.044	3.91 ± 0.74
ILH, *n* (%)	42 (5.6)	19 (1.6)
TC (mmol/L), mean ± SD	4.15 ± 0.063	4.07 ± 0.58
TG (mmol/L), median(Q1,Q3)*	1.16 (0.91, 1.41)	1.15 (1.03, 1.46)
HDL-C (mmol/L), mean ± SD*	0.92 ± 0.011	2.67 ± 0.08
LDL-C (mmol/L), mean ± SD*	2.69 ± 0.068	0.89 ± 0.66
Normal, *n* (%)	280 (37.5)	432 (37.4)
TC (mmol/L), mean ± SD	4.50 ± 0.019	4.54 ± 0.47
TG (mmol/L), median(Q1,Q3)*	0.92 (0.72, 1.21)	0.94 (0.73, 1.21)
HDL-C (mmol/L), mean ± SD*	1.33 ± 0.009	1.42 ± 0.25
LDL-C (mmol/L), mean ± SD*	2.60 ± 0.021	2.51 ± 0.53

*Statistically significant difference ($P < 0.05$) between men and women. SD, standard deviation; Q1, first quartile of the interquartile range; Q3, third quartile of the interquartile range. IHTG is defined as having TG ≥ 1.7 mmol/L or on medication and TC < 5.2 mmol/L; IHTC is defined as having TC ≥ 5.2 mmol/L or on medication and TG < 1.7 mmol/L; MHL is defined as having TG ≥ 1.7 mmol/L and TC ≥ 5.2 mmol/L; HDL-C (ILH) is defined as having HDL-C ≤ 1.0 mmol/L.

minor alleles of six SNPs (rs3890182 in *ABCA1*, rs4846914 and rs10127775 in *GALNT2*, rs2156552 in *LIPG-ACAA2*, rs1800775 in *CETP*, rs28927680 in *APOA1-C3-A4-A5-ZNF259-BUD13*) were associated with lower concentrations of HDL-C (Wallace *et al.* 2008; Teslovich *et al.* 2010). Inversely, the minor alleles of SNP rs17145738 in *BCL7B-TBL2-MLXIPL*, rs1077834 in *LIPC*, as well as SNP rs327 and rs331 in *LPL* were associated with higher concentrations of HDL-C (Deo *et al.* 2009; Teslovich *et al.* 2010). For LDL-C, the minor alleles of four SNPs (rs693 and rs676210 in *APOB*, rs4420638 in *APOE-C1-C4-C2*, rs2479409 and rs11583680 in *PCSK9*) show robust association with higher concentrations of LDL-C (Abifadel *et al.* 2003; Kathiresan *et al.* 2008; Chasman *et al.* 2009). Inversely, the minor alleles of five SNPs (rs599839 and rs646776 in the *CELSR2-PSRC1-SORT1*, rs12654264 and rs3846662 in the *HMGCR*, rs16996148 in *CILP2-PBX4*) were significantly associated with lower concentrations of LDL-C (Kathiresan *et al.* 2008; Wallace *et al.* 2008). For total cholesterol, three SNPs (rs4970834 in *CELSR2*, rs2479409 and rs11583680 in *PCSK9*) were associated with the concentrations of TC (Abifadel *et al.* 2003; Wallace *et al.* 2008; Teslovich *et al.* 2010). For triglycerides, the minor alleles of SNP rs693 in *APOB*, rs4846914 in *GALNT2*, rs780094 in *GCKR* and rs28927680 in *APOA1-C3-A4-A5-ZNF259-BUD13* show significant association with higher concentrations of TG (Kathiresan *et al.* 2008; Willer *et al.* 2008; Teslovich *et al.* 2010). Conversely, those of SNP rs17145738 in *BCL7B-TBL2-MLXIPL*, rs780092 in *GCKR*, rs327 and rs331 in LPL, rs17321515 in *TRIR1*, rs16996148 in *CILP2-PBX4* were associated with the lower concentrations of TG (Kathiresan *et al.* 2008; Willer *et al.* 2008; Deo *et al.* 2009; Teslovich *et al.* 2010; Kim *et al.* 2011). We selected 25 SNPs at 18 genetic loci and genotyped them in Chinese population. The final SNP set with minor allele frequency is > 0.01 and the *P* value of Hardy–Weinberg equilibrium (*P*_HWE) is > 0.001.

Genotyping

Venous blood was collected from each subject in an EDTA-containing tube. Genomic DNA was extracted from the blood by serial phenol–chloroform extraction and ethanol precipitation. SNP genotyping was performed by the dye terminator-based SNaPshot method, as previously described (Lu *et al.* 2010). All primers are listed in the table 2 in electronic supplementary material. All 25 SNPs at 18 genetic loci were genotyped. Genotyping success rate and accuracy were >98%, judged by random regenotyping of 10% of the samples in the subject group.

Statistical analysis

All statistical were analysed using SPSS ver. 20.0 (IBM Corp, Chendu, China). Continuous variables were presented as mean ± standard deviation (SD) or median (interquartile range) and categorical variables were presented as frequencies and proportions. The independent sample *t*-test or Mann–Whitney U test was used to investigate the relationship between continuous variables. The normal distributions of all quantitative variables were measured with the mean value and its SD, and with the Shapiro–Wilk test. However, none of the lipid levels were normally distributed according to the Shapiro–Wilk test. Therefore, to assess the influence of the covariates, multivariate analysis was employed with less-stringent normality criteria: the 99.10–99.84–100 rule, according to which about 99.10% of the values should fit within an interval of one SD, 99.84% in two SDs and 100% in three SDs. Among all variables measurements, the TG and glucose levels were not less-stringent normally distributed, so they were Napierian logarithm (ln) transformed for further statistical analysis. A standard χ^2 test was used to evaluate the Hardy–Weinberg equilibrium (HWE) and categorical variables. All the results were considered to be statistically significant with $P < 0.05$. The Bonferroni correction was used to adjust P values for multiple testing. Multivariate analysis was used to evaluate association of genetic loci with lipid and cholesterol phenotypes adjusted for the covariates (age, BMI and glucose levels). Logistic regression was applied to assess the association of genetic loci with risk of dyslipidemia by using possible covariates (age, gender, BMI, glucose levels and blood pressure). Independent associations among significant SNPs for different types of dyslipidemia were detected with multiple logistic regression analyses.

Results

In this study, we recruited a total of 1900 individuals, including 746 males and 1154 females with a mean age of 60.06 ± 13.21 and 59.61 ± 10.85 years, respectively. As there were significant differences of four lipid parameters and BMI between men and women groups, gender stratification analysis was conducted for each genetic locus. Basic characteristic of the study subjects is listed in table 1.

All 25 SNPs were genotyped, and two SNPs (rs28927680 and rs676210) were excluded due to the minor allele frequency (MAF) < 0.01 and P HWE < 0.001, respectively. The distributions of the rest of 23 SNP alleles were within the Hardy–Weinberg equilibrium. Association analysis results of lipid parameters with identified genetic loci in overall subjects are shown in table 3 in electronic supplementary material. Twelve SNPs located at nine genetic loci were significantly associated with one or more lipid traits (table 2). For LDL cholesterol, the minor alleles of five SNPs (rs599839 and rs646776 in *CELSR2-PSRC1-SORT1*, rs12654264 and rs3846662 in *HMGCR* and rs4970834 in *CELSR2*) were significantly associated with lower concentrations of LDL-C. Moreover, three SNPs (rs12654264, rs599839 and rs646776) have only showed association in women group, while the other two SNPs (rs3846662 and rs4970834) showed significant association within both men and women groups. For HDL cholesterol, five SNPs (rs3812316, rs327, rs331, rs1077834 and rs1800775) in *MLXIPL*, *LPL*, *LIPC* and *CETP* were associated with HDL-C. The minor alleles of two leading SNPs (rs327 and rs331) in the *LPL* gene were associated with lower concentrations of HDL-C. For total cholesterol, eight SNPs at six genetic loci were associated with the TC concentration. For triglycerides, the minor alleles of two SNPs (rs327 and rs331) in the *LPL* gene, rs17145738 in *BCL7B-TBL2-MLXIPL* and rs3812316 in *MLXIPL* were associated with lower concentrations of TG, which showed association in women group (table 2). After Bonferroni correction, several significant SNPs in *CELSR2*, *CELSR2-PSRC1-SORT1*, *HMGCR* and *LPL* still show significant association with corresponding phenotypes.

To investigate whether the 12 lipid-associated SNPs were related to dyslipidemia, we divided the 1900 participants into normal and four dyslipidemia phenotypes groups, and then evaluated the allele frequencies between normal group and each dyslipidemia group. After the association analysis, 10 SNPs significantly associated with different kinds of dyslipidemia which are shown in table 3. rs599839 and rs646776 in *CELSR2*, *PSRC1*, *SORT1*, as well as rs12654264 in *HMGCR* show significant association with the MHL in overall subjects ($P = 0.001$, OR = 0.406; $P = 0.017$, OR = 0.501; $P = 0.033$, OR = 0.779). Moreover, rs599839 and rs646776 in *CELSR2*, *PSRC1* and *SORT1* show more robust association with MLP in men group ($P = 0.007$, OR = 0.219; $P = 0.018$, OR = 0.26). However, rs17321515 in *TRIR1* just show weak association with the MHL and IHTC in women group ($P = 0.046$, OR = 1.376). Moreover, the minor allele of rs2479409 *PCSK9* and rs16996148 *CILP2*, *PBX4* is the risk factor for IHTC and ILH in men group ($P = 0.006$, OR = 1.63; $P = 0.013$, OR = 3.33). rs327 and rs331 in *LPL* are weakly

Table 2. SNPs significantly associated with lipid parameters.

SNP (minor allele)	Trait	Nearest genes	Position[a]	*P* HWE	MAF	Men		Women		Overall	
						P	B	*P*	B	*P*	B
rs4970834(T)	LDL-C	*CELSR2*	chr1: 109814880	0.92	0.039	0.024*	−0.303	0.006*	−0.282	4.49×10^{-4}**	−0.286
	TC					0.238	−0.167	0.01*	−0.284	0.008*	−0.236
rs599839(G)	LDL-C	*CELSR2*, *PSRC1*, *SORT1*	chr1: 109822166	0.505	0.065	0.116	−0.174	1.83×10^{-4}**	−0.292	1.5×10^{-5}**	−0.272
	TC					0.03*	−0.227	0.001**	−0.283	3.7×10^{-4}**	−0.242
rs646776(C)	LDL-C	*CELSR2*, *PSRC1*, *SORT1*	chr1: 109818530	0.67	0.084	0.18	−0.156	3.34×10^{-4}**	−0.297	3.5×10^{-5}**	−0.275
	TC					0.048*	−0.218	0.001**	−0.295	0.001	−0.248
rs12654264(A)	LDL-C	*HMGCR*	chr5: 74648603	0.055	0.492	0.051	−0.105	0.002*	−0.127	0.001	−0.103
	TC					0.277	−0.056	8.6×10^{-5}**	−0.169	1.4×10^{-5}**	−0.148
rs3846662(T)	LDL-C	*HMGCR*	chr5: 74651084	0.181	0.49	0.029*	−0.122	0.003*	−0.122	0.001	−0.109
	TC					0.126	−0.079	0.002*	−0.142	9.9×10^{-5}**	−0.138
rs17145738(T)	Ln TG[b]	*BCL7B*, *TBL2*, *MLXIPL*	chr7: 72982874			0.192	−0.076	0.063	−0.074	0.008*	−0.088
rs3812316(G)	HDL-C	*MLXIPL*	chr7: 73020337	0.283	0.089	0.215	0.042	0.119	0.038	0.034*	0.042
	Ln TG[b]					0.764	−0.018	0.009*	−0.11	0.023*	−0.079
rs327(G)	HDL-C	*LPL*	chr8: 19819536	0.323	0.189	0.003*	0.071	0.053	0.036	0.001**	0.049
	Ln TG[b]					0.102	−0.073	0.051	−0.062	0.017*	−0.062
rs331(A)	HDL-C	*LPL*	chr8: 19820405	0.31	0.184	0.012*	0.062	0.012*	0.045	1.19×10^{-4}**	0.056
	TC					0.112	0.113	0.309	0.056	0.048*	0.087
	Ln TG[b]					0.121	−0.069	0.033*	−0.065	0.01*	−0.065
rs17321515(A)	LDL-C	*TRIR1*	chr8: 126486409	0.1	431	0.086	0.094	0.017*	0.099	0.005*	0.093
	TC					0.846	0.011	0.002*	0.136	0.017*	0.086
rs1077834(C)	HDL-C	*LIPC*	chr15: 58723479	0.294	0.374	0.032*	0.043	0.032*	0.033	0.004*	0.035
	TC					0.303	0.059	0.007*	0.128	0.016*	0.089
rs1800775(A)	HDL-C	*CETP*	chr16: 56995236	0.284	0.458	0.129	−0.029	0.013*	−0.037	0.004*	0.034

*SNPs with *P* values <0.05; **SNPs with *P* values <0.05 after Bonferroni correction (corresponding *P* value × 12 <0.05). All calculations were made with a univariate analysis with covariates (age, BMI and glucose levels).

[a]Genomic position and chromosome in build 37.

[b]TG transformed by Napierian logarithm.

Table 3. SNPs significantly associated with different kinds of dyslipidemia.

SNP (minor allele)	Trait	Nearest gene	Position[a]	Men		Women		Overall	
				P	OR	*P*	OR	*P*	OR
rs599839(G)	MHL	*CELSR2, PSRC1, SORT1*	chr1: 109822166	0.007*	0.219	0.046*	0.516	0.001**	0.406
rs646776(C)	MHL	*CELSR2, PSRC1, SORT1*	chr1: 109818530	0.018*	0.26	0.23	0.67	0.017*	0.501
rs12654264(A)	MHL	*HMGCR*	chr5: 74648603	0.316	0.824	0.060	0.754	0.033*	0.779
rs17321515(A)	MHL	*TRIR1*	chr8: 126486409	0.797	0.949	0.044*	1.376	0.238	1.156
	IHTC			0.760	0.948	0.047*	1.274	0.164	1.147
rs2479409(A)	IHTC	*PCSK9*	chr1: 55504650	0.006*	1.631	0.965	0.995	0.108	0.175
rs16996148(T)	ILH	*CILP2, PBX4*	chr19: 19658472	0.013*	3.332	0.928	0.933	0.065	2.010
rs327(G)	ILH	*LPL*	chr8: 19819536	0.093	0.460	0.200	0.378	0.042*	0.456
rs331(A)	IHTG	*LPL*	chr8: 19820405	0.222	0.719	0.050	0.60	0.045*	0.694
rs4420638(G)	IHTG	*APOE-C1-C4-C2*	chr19: 45422946	0.30	1.316	0.004**	2.005	0.002**	1.712
rs28927680(G)	IHTG	*APOA1-C3-A4-A5, ZNF259, BUD13*	chr11: 116619073	0.626	0.907	0.035	0.661	0.066	0.778

[a]Genomic position and chromosome in build 37. IHTG is defined as having TG $\geq$ 1.7 mmol/L or on medication and TC $<$ 5.2 mmol/L; IHTC is defined as having TC $\geq$ 5.2 mmol/L or on medication and TG $<$ 1.7 mmol/L; MHL is defined as having TG $\geq$ 1.7 mmol/L and TC $\geq$ 5.2 mmol/L; ILH is defined as having HDL-C $\leq$ 1.0 mmol/L. *SNPs with *P* values $<$0.05; **SNPs with *P* values $<$0.05 after Bonferroni correction (corresponding *P* value $\times$ 10 $<$0.05). Overall the results were made with a logistic regression with covariates (age, gender, BMI, glucose levels and blood pressure), while the results of men or women were calculated by the logistic regression with covariates (age, BMI, glucose levels and blood pressure).

associated with IHL and IHTG ($P = 0.042$, OR $= 0.456$; $P = 0.045$, OR $= 0.694$). rs4420638 in *APOE-C1-C4-C2* and rs28927680 in *APOA1-C3-A4-A5*,*ZNF259*,*BUD13* show association with IHTG in women group and overall group.

To detect independent associations among the significant SNPs for dyslipidemia, multiple logistic regression analyses were conducted (table 4). For the phenotype of MHL, rs12654264 showed most robust and independent association with MHL in women group and overall group ($P = 0.001$, OR $= 0.576$; $P = 0.00$, OR $= 0.584$, respectively). For IHTC, rs2479409 is robustly and independently associated with IHTC in men group ($P = 0.003$, OR $=$ 1.816). For IHL, rs16996148 show strongest and independent association with IHL in men group and overall group ($P = 0.005$, OR $= 4.006$; $P = 0.011$, OR $= 2.78$). For IHTG, rs4420638 is robustly and independently associated with IHTG in women group and overall group ($P < 0.001$, OR $= 2.65$; $P = 0.001$, OR $= 1.91$).

Discussion

This study aims to identify associations of 25 identified SNPs with the blood lipid levels and different kinds of dyslipidemia in Chinese population. This is the first report, as far as we know that the most genetic loci involved in the lipid-related metabolic pathways were simultaneously studied with a relatively large group of Chinese (746 males and 1154 females). Our study has convincingly identified 12 of the 25 SNPs which were significantly associated with one or more blood lipid parameters, and 10 of the 12 associated SNPs were significantly associated with one or more types of dyslipidemia. Moreover, we further found four robust and independent association SNPs for four kinds of dyslipidemia.

In previous studies, 10 leading SNPs in *ABCA1*, *GALNT2*, *LIPG-ACAA2*, *CETP*, *APOA1-C3-A4-A5*, *ZNF259*, *BUD13* clusters, *BCL7B-TBL2-MLXIPL*, *LIPC* and *LPL* were associated with the blood concentrations of HDL-C (Wallace *et al.* 2008; Deo *et al.* 2009; Teslovich *et al.* 2010). In this study, we confirmed four SNPs in *LPL*, *LIPC* and *CETP* were associated with the HDL-C levels and linked a novel SNP in *MLXIPL* to it. Moreover, the minor alleles of two leading SNPs (rs327 and rs331 in *LPL*) significantly reduce the risk of ILH and IHTG, respectively. LPL is a triglyceride hydrolase hydrolysing the triglycerides in triglyceride-rich lipoproteins (TRLs: chylomicrons and VLDL) (Korn 1955a, b). LPL could play a role in stimulating clearance of remnant lipoproteins by the liver (Skottova *et al.* 1995). Recently, Smith *et al.* (2010) identified rs327 as the functional SNP in the 20 identified SNPs at *LPL* genetic locus.

For total cholesterol, three SNPs (rs4970834 in *CELSR2*, rs2479409 and rs11583680 in *PCSK9*) were associated with the concentrations of TC (Abifadel *et al.* 2003; Wallace

Table 4. Multiple logistic regression analysis of SNPs significantly associated with MLP of dyslipidemia in overall subjects.

Trait	SNP (minor allele)	Nearest gene	Men		Women		Overall	
			P	OR	*P*	OR	*P*	OR
MHL	rs599839(G)	*CELSR2*, *PSRC1*, *SORT1*	0.123	0.210	0.099	0.323	0.026*	0.289
	rs646776(C)	*CELSR2*, *PSRC1*, *SORT1*	0.631	0.604	0.453	1.704	0.787	1.17
	rs12654264(A)	*HMGCR*	0.598	0.892	0.001**	0.576	0.004**	0.684
	rs17321515(A)	*TRIR1*	0.742	1.07	0.006**	1.604	0.027*	1.34
IHTC	rs17321515(A)	*TRIR1*	0.304	0.8278	0.04	1.295	0.256	1.124
	rs2479409(A)	*PCSK9*	0.003**	1.816	0.70	0.95	0.186	1.15
ILH	rs16996148(T)	*CILP2*, *PBX4*	0.005**	4.066	0.678	1.39	0.011**	2.78
	rs327(G)	*LPL*	0.089	0.447	0.184	0.362	0.023**	0.408
IHTG	rs331(A)	*LPL*	0.731	0.078	0.038*	0.634	0.139	0.80
	rs4420638(G)	*APOE-C1-C4-C2*	0.467	1.232	<0.001**	2.651	0.001**	1.913
	rs28927680(G)	*APOA1-C3-A4-A5*, *ZNF259*, *BUD13*	0.423	0.842	0.154	0.73	0.071	0.762

*SNPs with *P* values <0.05; **SNPs with *P* values <0.05 after Bonferroni correction (corresponding *P* value × the number of SNPs in corresponding phenotype <0.05). MHL, mixed hyperlipidemia; Glu, glucose levels; BP, blood pressure. All calculations were calculated by a logistic regression with covariates (age, gender, BMI, glucose levels and blood pressure).

et al. 2008; Teslovich *et al.* 2010). In our study, seven novel SNPs in five novel genetic loci (*CELSR2-PSRC1-SORT1*, *HMGCR*, *LPL*, *TRIR1* and *LIPC*) showed significant association with TC. rs4970834 in *CELSR2* was also associated with TC, inversely, rs2479409 and rs11583680 in *PCSK9* were not so.

For triglycerides, SNP rs693 in *APOB*, rs4846914 in *GALNT2*, rs780094 in *GCKR*, rs28927680 in *APOA1-C3-A4-A5-ZNF259-BUD13*, rs17145738 in *BCL7B-TBL2-MLXIPL*, rs780092 in *GCKR*, rs327 and rs331 in *LPL*, rs17321515 in *TRIR1* and rs16996148 in *CILP2-PBX4* were associated with the lower concentrations of TG (Kathiresan *et al.* 2008; Willer *et al.* 2008; Deo *et al.* 2009; Teslovich *et al.* 2010; Kim *et al.* 2011). We confirmed that rs17145738 in *BCL7B-TBL2-MLXIPL*, as well as rs327 and rs331 in *LPL* were associated with TG, while the other SNPs were not so in Chinese population.

For LDL-C, previous studies found that nine SNPs (rs693 and rs676210 in *APOB*, rs4420638 in *APOE-C1-C4-C2*, rs2479409 and rs11583680 in *PCSK9*, rs599839 and rs646776 in *CELSR2-PSRC1-SORT1*, rs12654264 and rs3846662 in *HMGCR*, rs16996148 in *CILP2-PBX4*) were significantly associated with LDL-C (Abifadel *et al.* 2003; Kathiresan *et al.* 2008; Wallace *et al.* 2008). We validated four SNPs in *CELSR2-PSRC1-SORT1* and *HMGCR* associated with LDL-C, and discovered two novel associated SNPs (rs4970834 and rs17321515) in *CELSR2* and *TRIR1*. Further investigation suggests that the minor alleles of three SNPs in *CELSR2*, *CELSR2-PSRC1-SORT1* and *HMGCR* have significantly decreased the risk of MHL. And our results was consistent with the other replication study in a Japanese population (Nakayama *et al.* 2009).

For dyslipidemia, we found four SNPs showing robust and independent associations with four kinds of dyslipidemia. For the phenotype of MHL, rs12654264 in *HMGCR* (0.103 mmol/L per A allele) showed strong evidence of association with MHL after multiple testing corrections. Many previous studies have reported that rs12654264 is associated with trait of LDL-C in different populations (Hamrefors *et al.* 2010; Liu *et al.* 2011; Park *et al.* 2011; Taylor *et al.* 2013). For the phenotype of IHTC, the association between rs12654264 and IHTC in *PCSK9* proved to be gender-specific with significance observed only in males, but not in females. And rs16996148 is also gender-specific with significance observed only in males for ILH phenotype, which is consistent with previous study (Yan *et al.* 2011). For IHTG, rs4420638 show strong and independent association with rs4420638. rs4420638 showed strong evidence for association with LDL-C (Liu *et al.* 2011). Moreover, Huang *et al.* (2015) report that rs4420638 genotype AA is significantly associated with the concentrations of circulating HDL-C and APOA-I in CHD in Han Chinese males. As far as we know, we firstly discover that rs4420638 sex-specific with significance observed only in women for IHTG phenotype.

However, our study have some limitations. Since the impact of the environmental factors on lipid levels is important, our study lack the information on demographics, socioeconomic status, cigarette smoking, alcohol consumption and physical activity. Further analysis concerning the correlation between SNPs in candidate genes is warranted.

In conclusion, we first found four SNPs (rs12654264 in *HMGCR*; rs2479409 in *PCSK9*; rs16996148 in *CILP2*, *PBX4*; rs4420638 in *APOE-C1-C4-C2*) robustly and independently associate with four types of dyslipidemia. Thus, our results suggest that the genetic variants strongly influence one or more plasma lipid levels and the risk of dyslipidemia in Chinese population.

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