Int J Clin Exp Pathol 2017;10(3):2652-2674
www.ijcep.com /ISSN:1936-2625/IJCEP0044832

Original Article

AGBL4, PRL8 and *PCSK9* genetic variants and their interactions on dyslipidemia

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Received November 22, 2016; Accepted November 27, 2016; Epub March 1, 2017; Published March 15, 2017

Abstract: This study was designed to comprehensively illuminate the genetic susceptibility to dyslipidemia on the ATP/GTP binding protein-like 4 (AGBL4), low-density lipoprotein (LDL) receptor related protein 8 (LRP8) and proprotein convertase subtilisin-like kexin type 9 (PCSK9) gene cluster regions. Genotypes of 12 single nucleotide polymorphisms (SNPs) were identified in a total of 2552 individuals (1020, hypercholesterolemia (HTC); 740, hypertriglyceridemia (HTG); 673, hyper-LDL cholesterol (HLDL-C); and 1177, hyper-apolipoproteinB100 (HApoB100), some participants overlapped more than one kind of dyslipidemia). Consequently, we confirmed previously observed significant associations between cardiometabolic risk, dyslipidemia, and SNPs in the *AGBL4* (rs320018 for HTG, rs320017 for HLDL-C, rs320017 and rs320018 for HApoB100), *LRP8* (rs1288521 for HApoB100) and *PCSK9* (rs584626 and rs585131 for HTC, rs533375 for HTG, rs540796 for HLDL-C, and rs533375 for HApoB100). Furthermore, we elucidated distinct effects of the *AGBL4*, *LRP8* and *PCSK9* interactions towards dyslipidemia (G-G-A-A-C-G-T-T-C-A-A-G for HTC; A-A-G-G-A-G-C-C-C-A-A-G and G-G-A-A-C-G-T-T-T-A-A-G for HTG; A-A-G-A-C-G-T-T-C-A-A-G, A-A-G-G-A-G-C-C-C-A-A-G and G-G-A-A-C-G-T-T-T-G-G-A for HLDL-C; and A-A-G-A-C-G-T-C-C-A-A-G for HApoB100). These findings suggest that integrative *AGBL4*, *LRP8* and *PCSK9* genetic variants and their interactions may significantly modify the risk of dyslipidemia, depending on effects of serum lipid levels.

Keywords: ATP/GTP binding protein-like 4 gene, low-density lipoprotein receptor related protein 8 gene, proprotein convertase subtilisin-like kexin type 9 gene, dyslipidemia

Introduction

There is long-standing, well-documented and robust evidence showing dyslipidemia is an independent risk factor for atherosclerotic cardiovascular disease (ASCVD). Although the primary target of therapy for prevention of ASCVD was mainly focused on reducing the plasma low-density lipoprotein cholesterol (LDL-C) levels recommend by current guidelines from ESC/EAS [1], lowering plasma total cholesterol (TC) [2], triglyceride (TG) [3] and apolipoproteinB100 (ApoB100) [4] were found to be more beneficial than lowering LDL-C alone for ASCVD. Dyslipidemia represents overlapping complex disease attributed to genetic and environmental factors, while the estimated heritability for dyslipidemia is quite high, approximately 40-60% [5].

Despite hundreds of genetic variants hit from very large genome-wide association studies (GWASs) [6], epigenome-wide association studies (EWASs) [7] and transcriptome-wide association studies (TWASs) [8] on dyslipidemia, a large portion of single nucleotide polymorphisms (SNPs) that appear to influence dyslipidemia, including the ATP/GTP binding protein-like 4 gene (*AGBL4*; Gene ID: 84871; MIM: 616476; formerly known as *CCP6*; location: 1p33, exon count: 17) (http://www-ncbi-nlm-nih-gov.ezp-prod1.hul.harvard.edu/gene/), the LDL receptor related protein 8 gene (*LRP8*; Gene ID: 7804; MIM: 602600; formerly known as *MCI1*, *LRP-8*, *APOER2* and *HSZ75190*; location: 1p34, exon count: 22) and the proprotein convertase subtilisin-like kexin type 9 gene (*PCSK9*; Gene ID: 255738; MIM: 607786; formerly known as *FH3*, *PC9*, *NARC1*, *LDLCQ1*, *NARC-1* and

HTCOLA3; location: 1p32.3, exon count: 14) remains unexplained [9-11].

Population-based association of *AGBL4*, *PRL8* and *PCSK9* variants with lipid-related traits has been reported previously [12], while comprehensive analyses of *AGBL4*, *PRL8* and *PCSK9* variants and their possible gene-gene interaction on dyslipidemia have never been detected. Therefore, this study was performed to test the hypothesis that (i) *AGBL4* (rs320017, rs320018 and rs320019), *LRP8* (rs6694764, rs1288519, rs585131, rs1288520 and rs1288521) and *PCSK9* (rs533375, rs584626, rs585131 and rs540796) variants associate with dyslipidemia, comprising HTC, HTG, HLDL-C and HApoB100 (in our laboratory, ApoB level mainly means ApoB100 level); (ii) their interactions of the detected variants involved in mechanisms of dyslipidemia; and (iii) the possible gene-gene interaction models among these variants are useful for identifying more precise and distinct susceptible signals of dyslipidemia, comparing with single-locus test.

Materials and methods

Ethics statements

The study protocol was approved by the Ethics Committee of the First Affiliated Hospital, Guangxi Medical University. Written informed consent for all the participants is obtained as per the guidelines. This study was carried out following the rules of the Declaration of Helsinki of 1975 (http://www.wma.net/en/30publications/10policies/b3/), revised in 2008.

Subjects

The participants were recruited from three islands: Wanwei, Wutou and Shanxin of Dongxing City, Guangxi Zhuang Autonomous Region, China. A total of 2552 participants were randomly selected from our stratified, randomized samples [13]. There were 1866 dyslipidemic individuals, comprising hypercholesterolemia (HTC > 5.17 mmol/l), hypertriglyceridemia (HTG > 1.70 mmol/l), high low-density lipoprotein cholesterol (HLDL-C > 3.10 mmol/l) or high apolipoprotein B100 (HApoB100 > 1.05 g/L) participants and 686 normolipidemic subjects (TC ≤ 5.17, TG ≤ 1.70, LDL-C ≤ 3.10 mmol/l and ApoB100 ≤ 1.05 g/L) [14-17], aged 18-80 years [18-20]. Some participants overlap more than one kind of dyslipidemia (HTC, HTG, HLDL-C and/or HApoB100). Then, within the dyslipidemia individuals to assess the association of variants with risk of HTC, HTG, LDL-C and ApoB100 separately. The participants with a history of coronary artery disease, stroke, diabetes, hyper- or hypo-thyroids, and chronic hepatic and renal disease, as well as a history of taking lipid modulating medications such as statins or fibrates were excluded [21].

Epidemiological survey

The epidemiological survey was carried out by using internationally standardized methods and following a common protocol [22]. Information on demographics, socioeconomic status, lifestyle, past medical history and family disease history was collected by using standardized questionnaires. The intake of alcohol was quantified as the number of liangs (about 50 g) of rice wine, corn wine, rum, beer or liquor consumed during the preceding 12 months. Alcohol consumption was categorized into groups of grams of alcohol per day: 0 (non-drinkers), ≤ 25 and > 25 [23]. Smoking status was categorized into the groups of cigarettes per day: 0 (non-smokers), ≤ 20 and > 20 [24]. The methods of blood pressure, height, weight and waist circumference measurements have been described in the previous studies. Body mass index (BMI, kg/m^2) was calculated from the height and weight measurements and was categorized into four groups: under weight (BMI < 18.5), normal weight (18.5 ≤ BMI < 24.9), overweight (25 ≤ BMI < 29.9), class I obesity (30 ≤ BMI < 34.9), class II obesity (35 ≤ BMI < 39.9), class III obesity (40 < BMI) [25]. Waist circumference was categorized into groups including normal (waist circumference ≤ 40 in, male; waist circumference ≤ 35 in, female) and central obesity (waist circumference > 40 in, male; waist circumference > 35 in, female) [26].

Laboratory biochemical measurement

Lipid variables and fasting glucose levels were measured by standardized methods in blood samples obtained after a 12-h fast. The normal values of serum TC, TG, HDL-C, LDL-C, ApoA1 and ApoB100 (also called ApoB in our laboratory) levels and the ratio of ApoA1 to ApoB100 in our Clinical Science Experiment Centre were 3.10-5.17, 0.56-1.70, 1.16-1.42, 2.70-

3.10 mmol/l, 1.20-1.60, 0.80-1.05 g/l and 1.00-2.50; respectively. According to fasting plasma glucose (FPG) levels, the subjects were categorized into three groups: normal glucose levels (< 6.1 mmol/L), impaired fasting glucose (≥ 6.1 and < 7) and diabetes mellitus (≥ 7 mmol/L) [27].

DNA isolation and genotyping

Genomic DNA was isolated from peripheral blood leucocytes using the phenol-chloroform method [28, 29]. Genotyping of the detected variants was performed by polymerase chain reaction (PCR) and then direct sequenced by Sanger sequencing technology using ABI Prism 3100 (Applied Biosystems, International Equipment Trading Ltd., Vernon Hills, IL, USA) in Shanghai Sangon Biological Engineering Technology & Services Co. Ltd., Shanghai China.

Statistical methods

A statistical software package SPSS 21.0 (SPSS Inc., Chicago, IL, USA) was used to analyze the data. The quantitative variables were presented as the mean ± S.D. for those, that are normally distributed, and the medians and interquartile ranges for TG, which is not normally distributed. General characteristics between the two groups were compared by the Student's unpaired *t*-test. The allele frequency and genotype distribution, as well as haplotype frequency between the groups were analyzed by the Chi-squared test; Hardy-Weinberg equilibrium, pair-wise linkage disequilibria and haplotype frequencies among the variants were analyzed using Haploview (version 4.2; Broad Institute of MIT and Harvard). The association between the genotypes and serum lipid phenotypes was tested by analysis of covariance (ANCOVA). Unconditional logistic regression was used to assess the correlation between the risk of dyslipidemia and genotypes (the minor allele non-carrier = 1, the minor allele carrier = 2). Age, sex, BMI, smoking and alcohol consumption were adjusted for the statistical analysis. Two-sided $P < 0.05$ was considered statistically significant.

The inter-locus interaction was analyzed by generalized multifactor dimensionality reduction (GMDR) method, using GMDR software. The cross-validation consistency score provides the degree of consistency when the selected interaction is identified as the best model among all possibilities considered. The testing balanced accuracy provides the degree of interaction, which accurately predicts the case-control status with scores between 0.50 (indicating that the model predicts no better than the chance) and 1.00 (indicating perfect prediction). A sign test or a per-variant test provides *P*-value for predicting accuracy to measure the significance of an identified model. The best model is selected as the combination of marker with maximum cross-validation consistency and minimum prediction error.

Results

Clinical characteristics

Clinical characteristics of the 2552 individuals (some of them overlapped more than one kind of dyslipidemia) in this study are summarized in **Table 1**.

Genotype and allele frequencies

The genotype and allele frequencies of the *AGBL4*, *LRP8* and *PCSK9* variants are summarized in **Table 2**. The genotype frequency of the *AGBL4* rs320017, *AGBL4* rs320018, *AGBL4* rs320019, *PCSK9* rs585131 and *PCSK9* rs540796 and the allele frequency of the *AGBL4* rs320017-G, *PCSK9* rs585131-G and *PCSK9* rs540796-A were significantly different between the HTC and non-HTC individuals ($P < 0.05$-0.001). Likewise, the genotype frequency of the *AGBL4* rs320017, *AGBL4* rs320018, *AGBL4* rs320019, *LRP8* rs6694764, *LRP8* rs1288519, *LRP8* rs872315, *LRP8* rs1288520, *LRP8* rs1288521, *PCSK9* rs533375, *PCSK9* rs584626, *PCSK9* rs585131 and *PCSK9* rs540796 and the allele frequency of the *AGBL4* rs320017-G, *AGBL4* rs320018-G, *AGBL4* rs320019-A, *LRP8* rs6694764-A, *LRP8* rs1288519-C, *LRP8* rs872315-A, *LRP8* rs1288520-T, *LRP8* rs1288521-T, *PCSK9* rs533375-T, *PCSK9* rs584626-G, *PCSK9* rs585131-G and *PCSK9* rs540796-A were significantly different between the HTG and non-HTG individuals ($P < 0.05$-0.001). Furthermore, the genotype frequency of the *AGBL4* rs320017, *AGBL4* rs320018, *LRP8* rs6694764, *LRP8* rs1288519, *LRP8* rs1288520, *LRP8* rs1288521, *PCSK9* rs533375, *PCSK9* rs584626, *PCSK9* rs585131 and *PCSK9* rs540796 and the allele fre-

Table 1. Clinical characteristics among the dyslipidemia individuals

Characteristics	HTC	HTG	HLDL-C	HApoB100
Number	1020	740	673	1177
Gender (Male/Female) [n (%)]	502 (49.2)/518 (50.8)	368 (49.7)/372 (50.3)	314 (46.7)/359 (53.3)	588 (50.0)/589 (50.0)
Age (years)	59.47 ± 13.03	56.44 ± 12.90	58.48 ± 12.78	57.85 ± 12.68
Younger adults < 40 years of age	50 (4.9)	55 (7.4)	35 (5.2)	62 (5.3)
Adults 40-75 years of age	833 (81.7)	613 (82.8)	562 (83.5)	985 (83.7)
Elderly adults > 75 years of age	137 (13.4)	72 (9.7)	76 (11.3)	130 (11.0)
Race/Ethnicity (Jing/Han) [n (%)]	593 (58.1)/427 (41.9)	404 (54.6)/336 (45.4)	344 (51.1)/329 (48.9)	607 (51.6)/570 (48.4)
Height (cm)	157.26 ± 7.84	158.58 ± 8.59	157.61 ± 8.65	158.14 ± 8.09
Weight (kg)	58.10 ± 10.31	61.08 ± 10.93	56.64 ± 9.77	59.47 ± 10.29
Body mass index (kg/m^2)	23.41 ± 3.28	24.21 ± 3.36	22.82 ± 3.07	23.71 ± 3.32
BMI < 18.5 (under weight)	62 (6.1)	33 (4.5)	42 (6.2)	62 (5.3)
BMI 18.5-24.9 (normal weight)	676 (66.3)	419 (56.6)	488 (72.5)	751 (63.8)
BMI 25-29.9 (over weight)	249 (24.4)	252 (34.1)	126 (18.7)	318 (27.0)
BMI 30-34.9 (class I obese)	30 (2.9)	34 (4.6)	16 (2.4)	39 (3.3)
BMI 35-39.9 (class II obese)	3 (0.3)	2 (0.3)	1 (0.1)	7 (0.6)
BMI ≥ 40 (class III obese [extreme obesity])	0 (0)	0 (0)	0 (0)	0 (0)
Waist circumference(cm)	79.89 ± 9.46	82.99 ± 9.12	78.08 ± 9.09	80.92 ± 9.20
Male (Waist circumference ≤ 102 [40 in])	498 (99.2)	366 (99.5)	312 (99.4)	584 (99.3)
Male (Waist circumference > 102 [40 in])	4 (0.8)	2 (0.5)	2 (0.6)	4 (0.7)
Female (Waist circumference ≤ 88 [35 in])	445 (86.4)	306 (82.3)	324 (90.5)	493 (84.1)
Female (Waist circumference > 88 [35 in])	70 (13.6)	66 (17.7)	34 (9.5)	93 (15.9)
Systolic blood pressure (mmHg)	133.85 ± 20.87	134.68 ± 19.86	131.98 ± 20.71	133.91 ± 19.74
Diastolic blood pressure (mmHg)	81.86 ± 10.90	83.20 ± 10.64	80.24 ± 10.75	82.02 ± 10.54
Pulse pressure (mmHg)	51.99 ± 16.90	51.48 ± 16.01	51.74 ± 16.39	51.88 ± 15.91
SBP < 120 & DBP < 80	147 (14.4)	72 (9.7)	116 (17.2)	141 (12.0)
Have prehypertension (SBP 120-139 or DBP80-89)	415 (40.7)	326 (44.1)	289 (42.9)	487 (41.4)
Have stage 1 hypertension (SBP 140-159 or DBP90-99)	260 (25.5)	205 (27.7)	163 (24.2)	358 (30.4)
Have stage 2 hypertension (SBP 160-179 or DBP100-19)	141 (13.8)	91 (12.3)	74 (11.0)	144 (12.2)
Have stage 3 hypertension (SBP ≥ 180 or DBP ≥ 110)	57 (5.6)	46 (6.2)	31 (4.6)	47 (4.0)
Have isolated systolic hypertension (SBP ≥ 140 & BP < 90)	172 (16.9)	116 (15.7)	103 (15.3)	193 (16.4)
Tobacco smoking [n (%)]				
Nonsmoker	819 (80.3)	536 (72.4)	541 (80.4)	938 (79.7)
Light to moderate (≤ 20 Tobacco smoking/day)	49 (4.8)	31 (4.2)	25 (3.7)	44 (3.7)
Severe (> 20 Tobacco smoking/day)	152 (14.9)	173 (23.4)	107 (15.9)	195 (16.6)

Alcohol consumption [n (%)]				
Nondrinker	728 (71.4)	527 (71.2)	505 (75.0)	856 (72.7)
Light to moderate (≤ 25 g/day)	114 (11.2)	59 (8.0)	68 (10.1)	105 (8.9)
Severe(> 25 g/day)	178 (17.5)	154 (20.8)	100 (14.9)	216 (18.4)
Blood glucose level (mmol/L)	6.95 ± 1.55	7.04 ± 1.71	6.66 ± 1.43	6.72 ± 1.52
Glucose < 6.1 mmol/L	211 (20.7)	179 (24.2)	242 (36.0)	373 (31.7)
Have impaired fasting glucose (≥ 6.1 & < 7 mmol/L)	384 (37.6)	279 (37.7)	236 (35.1)	439 (37.3)
Have diabetes mellitus (≥ 7 mmol/L)	425 (41.7)	282 (38.1)	195 (29.0)	365 (31.0)
Total cholesterol (mmol/L)	5.89 ± 0.57	5.32 ± 0.92	5.05 ± 0.88	5.45 ± 0.77
Triglyceride (mmol/L)	1.53 (1.20)	2.19 (1.90)	1.33 (1.05)	1.59 (1.23)
High-density lipoprotein cholesterol (mmol/L)	1.82 ± 0.50	1.77 ± 0.49	2.01 ± 0.52	1.81 ± 0.49
Low-density lipoprotein cholesterol (mmol/L)	2.88 ± 0.42	2.85 ± 0.41	3.37 ± 0.23	2.87 ± 0.43
Apolipoprotein (Apo) A1 (g/L)	1.36 ± 0.26	1.27 ± 0.21	1.34 ± 0.22	1.34 ± 0.24
ApoB100 (g/L)	1.18 ± 0.25	1.15 ± 0.24	1.07 ± 0.26	1.26 ± 0.17
ApoA1/ApoB100	1.20 ± 0.33	1.16 ± 0.34	1.33 ± 0.41	1.08 ± 0.21

HTC: hypercholesterolemia; HTG: hypertriglyceridemia; HLDL-C: hyper low-density lipoprotein cholesterol; HApoB100: hyperapolipoprotein (Apo) B100.

Table 2. *AGBL4*, *LRP8* and *PCSK9* variants on dyslipidemia (with *P*-value < 0.05)

SNP	Genotype	Genotype distribution, n (%)			OR [95% CI]	*P*-value	Dyslipidemia
		Cases (n = 1020)	Controls (n = 1532)	*P*-value			
AGBL4 rs320017 A > G	AA	665 (65.20)	939 (61.29)				
	AG/GG	355 (34.80)	593 (38.71)	0.046	0.672 [0.435, 1.037]	0.073	HTC
	MAF	398 (19.51)	672 (21.93)	0.037			
	HWE (*P*)	0.405	0.428				
AGBL4 rs320018 A > G	AA	660 (64.71)	929 (60.64)				
	AG/GG	360 (35.29)	603 (39.36)	0.038	0.660 [0.427, 1.020]	0.062	HTC
	MAF	407 (19.95)	679 (22.16)	0.059			
	HWE (*P*)	0.210	0.910				
AGBL4 rs320019 G > A	GG	668 (65.49)	935 (61.03)				
	AG/AA	352 (34.51)	597 (38.97)	0.022	0.561 [0.246, 1.282]	0.170	HTC
	MAF	401 (19.66)	672 (21.93)	0.051			
	HWE (*P*)	0.057	0.845				
PCSK9 rs585131 A > G	AA	934 (91.57)	1356 (88.51)				
	AG/GG	86 (8.43)	176 (11.49)	0.013	0.840 [0.106, 6.680]	0.840	HTC
	MAF	88 (4.31)	181 (5.91)	0.013			
	HWE (*P*)	0.938	0.874				
PCSK9 rs540796 G > A	GG	922 (90.39)	1329 (86.75)				
	AG/AA	98 (9.61)	203 (13.25)	0.005	0.034 [0.006, 0.191]	1.288E-04	HTC
	MAF	104 (5.10)	207 (6.76)	0.015			
	HWE (*P*)	0.030	0.225				
AGBL4 rs320017 A > G	AA	418 (56.49)	1186 (65.45)				
	AG/GG	322 (43.51)	626 (34.55)	2.106E-05	2.216 [0.303, 16.202]	0.433	HTG
	MAF	368 (24.86)	702 (19.37)	1.213E-05			
	HWE (*P*)	0.961	0.228				
AGBL4 rs320018 A > G	AA	420 (56.76)	1169 (64.51)				
	AG/GG	320 (43.24)	643 (35.49)	2.440E-04	0.063 [0.005, 0.851]	0.037	HTG
	MAF	367 (24.78)	719 (19.84)	8.618E-05			
	HWE (*P*)	0.768	0.490				
AGBL4 rs320019 G > A	GG	416 (56.22)	1187 (65.51)				
	AG/AA	324 (43.78)	625 (34.49)	1.049E-05	5.728 [0.658, 49.894]	0.114	HTG
	MAF	372 (25.14)	701 (19.34)	4.070E-06			
	HWE (*P*)	0.807	0.217				
LRP8 rs6694764 G > A	GG	222 (30.00)	648 (35.76)				
	AG/AA	518 (70.00)	1164 (64.23)	0.005	0.877 [0.194, 3.965]	0.865	HTG
	MAF	669 (45.20)	1452 (40.07)	0.001			
	HWE (*P*)	0.976	0.778				
LRP8 rs1288519 A > C	AA	232 (31.35)	677 (37.36)				
	AC/CC	508 (68.65)	1135 (62.64)	0.004	1.005 [0.153, 6.607]	0.996	HTG
	MAF	657 (44.39)	1414 (39.02)	3.883E-04			
	HWE (*P*)	0.637	0.756				
LRP8 rs872315 G > A	GG	684 (92.43)	1720 (94.92)				
	AG/AA	56 (7.57)	92 (5.08)	0.015	0.752 [0.234, 2.421]	0.633	HTG
	MAF	62 (4.19)	92 (2.54)	0.002			
	HWE (*P*)	1.66E-05	0.268				
LRP8 rs1288520 C > T	CC	237 (32.03)	696 (38.41)				
	CT/TT	503 (67.97)	1116 (61.59)	0.002	0.567 [0.074, 4.321]	0.584	HTG
	MAF	662 (44.73)	1412 (38.96)	1.410E-04			
	HWE (*P*)	0.104	0.039				
LRP8 rs1288521 C > T	CC	253 (34.19)	791 (43.65)				
	CT/TT	487 (65.81)	1021 (56.35)	1.023E-05	1.106 [0.308, 3.964]	0.877	HTG
	MAF	619 (41.82)	1258 (34.71)	1.746E-06			
	HWE (*P*)	0.700	0.053				
PCSK9 rs533375 C > T	CC	493 (66.62)	1356 (74.83)				
	CT/TT	247 (33.38)	456 (25.17)	2.510E-05	7.578 [2.685, 21.384]	1.301E-04	HTG
	MAF	283 (19.12)	498 (13.74)	1.270E-06			

	HWE (*P*)	0.034	0.123				
PCSK9 rs584626 A > G	AA	643 (86.89)	1635 (90.23)				
	AG/GG	97 (13.11)	177 (9.77)	0.013	0.053 [0.002, 1.824]	0.104	HTG
	MAF	109 (7.36)	177 (4.88)	4.712E-04			
	HWE (*P*)	1.68E-05	0.029				
PCSK9 rs585131 A > G	AA	646 (87.30)	1644 (90.73)				
	AG/GG	94 (12.70)	168 (9.27)	0.010	13.197 [0.432, 403.079]	0.139	HTG
	MAF	101 (6.82)	168 (4.64)	0.001			
	HWE (*P*)	0.040	0.039				
PCSK9 rs540796 G > A	GG	632 (85.41)	1619 (89.35)				
	AG/AA	108 (14.59)	193 (10.65)	0.005	1.135 [0.190, 6.783]	0.890	HTG
	MAF	118 (7.97)	193 (5.33)	3.337E-04			
	HWE (*P*)	0.008	0.017				
AGBL4 rs320017 A > G	AA	400 (59.44)	1204 (64.08)				
	AG/GG	276 (40.56)	675 (35.92)	0.024	5.709 [1.016, 32.059]	0.048	HLDL-C
	MAF	309 (22.96)	761 (20.25)	0.036			
	HWE (*P*)	0.908	0.201				
AGBL4 rs320018 A > G	AA	397 (58.99)	1192 (63.44)				
	AG/GG	276 (41.01)	687 (36.56)	0.041	0.438 [0.059, 3.246]	0.419	HLDL-C
	MAF	308 (22.88)	778 (79.30)	0.094			
	HWE (*P*)	0.479	0.141				
LRP8 rs6694764 G > A	GG	205 (30.46)	665 (35.39)				
	AG/AA	468 (69.54)	1214 (64.61)	0.021	1.412 [0.418, 4.765]	0.579	HLDL-C
	MAF	594 (44.13)	1527 (40.63)	0.025			
	HWE (*P*)	0.428	0.791				
LRP8 rs1288519 A > C	AA	217 (32.24)	692 (36.83)				
	AC/CC	456 (67.76)	1187 (63.17)	0.033	0.349 [0.063, 1.943]	0.230	HLDL-C
	MAF	581 (43.16)	1490 (39.65)	0.024			
	HWE (*P*)	0.951	0.463				
LRP8 rs1288520 C > T	CC	220 (32.69)	713 (37.95)				
	CT/TT	453 (67.31)	1166 (62.05)	0.015	1.191 [0.226, 6.278]	0.837	HLDL-C
	MAF	583 (43.31)	1491 (39.68)	0.020			
	HWE (*P*)	0.557	0.005				
LRP8 rs1288521 C > T	CC	251 (37.30)	793 (42.20)				
	CT/TT	422 (62.70)	1086 (57.80)	0.026	2.340 [0.843, 6.495]	0.103	HLDL-C
	MAF	537 (39.90)	1340 (35.66)	0.006			
	HWE (*P*)	0.205	0.129				
PCSK9 rs533375 C > T	CC	467 (69.39)	1382 (73.55)				
	CT/TT	206 (30.61)	497 (26.45)	0.038	1.092 [0.507, 2.353]	0.823	HLDL-C
	MAF	223 (16.57)	558 (14.85)	0.133			
	HWE (*P*)	0.681	0.000				
PCSK9 rs584626 A > G	AA	586 (87.07)	1692 (90.05)				
	AG/GG	87 (12.93)	187 (9.95)	0.032	0.542 [0.073, 4.008]	0.548	HLDL-C
	MAF	90 (6.69)	196 (5.22)	0.044			
	HWE (*P*)	0.996	0.070				
PCSK9 rs585131 A > G	AA	588 (87.37)	1702 (90.58)				
	AG/GG	85 (12.63)	177 (9.42)	0.019	0.404 [0.061, 2.679]	0.348	HLDL-C
	MAF	87 (6.46)	182 (4.84)	0.022			
	HWE (*P*)	0.605	0.767				
PCSK9 rs540796 G > A	GG	573 (85.14)	1678 (89.30)				
	AG/AA	100 (14.86)	201 (10.70)	0.004	7.050 [1.552, 32.017]	0.011	HLDL-C
	MAF	103 (7.65)	208 (5.53)	0.005			
	HWE (*P*)	0.608	0.583				
LRP8 rs1288521 C > T	CC	457 (38.83)	587 (42.69)				
	CT/TT	720 (61.17)	788 (57.31)	0.048	0.357 [0.137, 0.933]	0.036	HApoB100
	MAF	881 (37.43)	996 (36.22)	0.372			
	HWE (*P*)	0.631	0.001				

MAF, minor allele frequency; HWE, Hardy-Weinberg equilibrium; AGBL4, ATP/GTP binding protein-like 4; LRP8, LDL receptorrelated protein 8; PCSK9, Proprotein convertase subtilisin/kexin type 9; OR, odds ratio. HTC, hypercholesterolemia; HTG, hypertriglyceridemia; HLDL-C, hyper low-density lipoprotein cholesterol; HApoB100, hyper apolipoprotein (Apo) B100.

quency of the *AGBL4* rs320017-G, *LRP8* rs6694764-A, *LRP8* rs1288519-C, *LRP8* rs1288520-T, *LRP8* rs1288521-T, *PCSK9* rs584626-G, *PCSK9* rs585131-G and *PCSK9* rs540796-A were significantly different between the HLDL-C and non-HLDL-C individuals ($P < 0.05$-0.001). Moreover, the genotype frequency of the *LRP8* rs1288521 was significantly different between HApoB100 and non-HApoB100 ($P < 0.05$). There was no difference in allele frequency between HApoB100 and non-HApoB100 ($P > 0.05$ for all).

Single-variant on dyslipidemia

Figure 1 depicts the association between the *AGBL4*, *LRP8* and *PCSK9* genetic variants and serum lipid variables in the HTC and non-HTC individuals. The levels of TC (rs585131), TG (rs320017, rs1288519, rs872315, rs533375, rs584626 and rs585131), ApoA1 (rs6694764, rs1288519, rs1288520 and rs1288521), ApoB100 (rs872315) and the ratio of ApoA1 to ApoB100 (rs6694764, rs1288519 and rs1288520) in the HTC individuals were different between the genotypes ($P < 0.05$-0.001), whereas the levels of TG (rs320017, rs320018, rs320019, rs6694764, rs1288519, rs1288520, rs1288521, rs533375, rs584626, rs585131 and rs540796), HDL-C (rs584626) and ApoB100 (rs320019) in the non-HTC individuals were different between the genotypes ($P < 0.05$-0.001).

Figure 2 depicts the association between the genotypes and serum lipid phenotypes in the HTG and non-HTG individuals. The levels of TC (rs320017, rs320018 and rs320019), TG (rs320017, rs320019, rs872315, rs1288521 and rs533375), HDL-C (rs6694764), LDL-C (rs872315, rs585131 and rs540796), ApoA1 (rs320017, rs320018, rs320019, rs6694764, rs1288519, rs1288520 and rs1288521), ApoB100 (rs872315 and rs533375) and the ratio of ApoA1 to ApoB100 (rs533375) in the HTG individuals were different between the genotypes ($P < 0.05$-0.001); whereas the levels of HDL-C (rs872315, rs584626, rs585131 and rs540796) in the non-HTG individuals were different between the genotypes ($P < 0.05$-0.001).

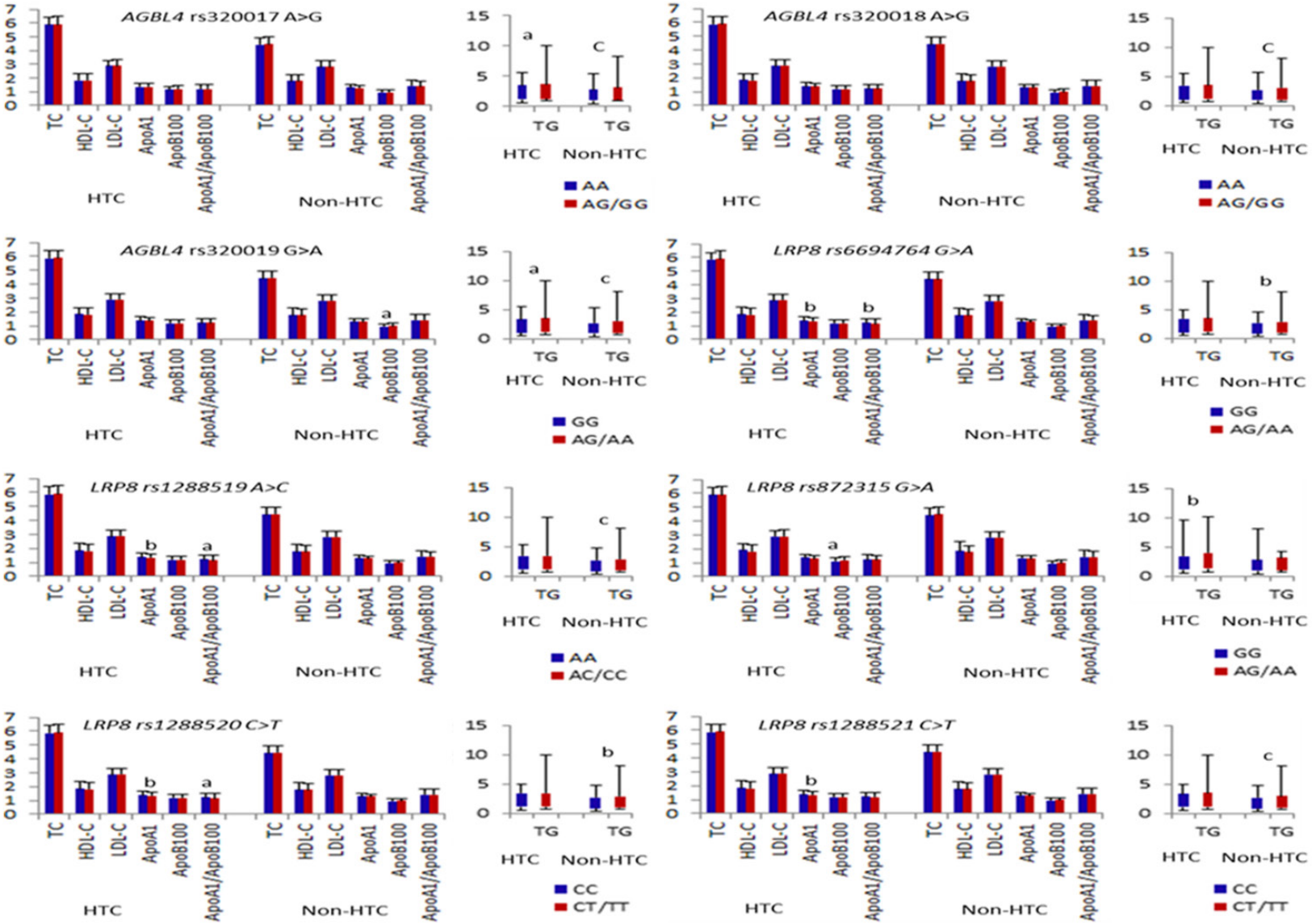
Figure 3 depicts the association between the genotypes and serum lipid phenotypes in the HLDL-C and non-HLDL-C individuals. The levels of HDL-C (rs872315, rs584626 and rs585131), LDL-C (rs320017, rs320018 rs320019, and rs1288521) and ApoA1 (rs6694764, rs1288519, rs1288520 and rs1288521) in the HLDL-C individuals were different between the genotypes ($P < 0.05$-0.001); whereas the levels of TG (rs320017, rs320018, rs320019, rs6694764, rs1288519, rs872315, rs1288520, rs1288521, rs533375, rs584626, rs585131 and rs540796), HDL-C (rs533375) and ApoA1 (rs584626 and rs585131) in the non-HLDL-C individuals were different between the genotypes ($P < 0.05$-0.001).

Figure 4 depicts the association between the genotypes and serum lipid phenotypes in the HApoB100 and non-HApoB100 individuals. The levels of TC (rs320017, rs320018 and rs320019), TG (rs320017, rs320018, rs320019, rs6694764, rs1288519, rs1288520, rs1288521, rs533375, rs585131 and rs540796), HDL-C (rs6694764), ApoA1 (rs6694764, rs1288519, rs1288520, and rs1288521), ApoB100 (rs320017, rs320018 and rs320019, rs6694764, rs872315 and rs533375) and the ratio of ApoA1 to ApoB100 (rs320018, rs6694764, rs1288519, rs1288520, and rs1288521) in the HApoB100 individuals were different between the genotypes ($P < 0.05$-0.001); whereas the levels of TG (rs320017, rs320018, rs320019, rs6694764, rs1288519, rs872315, rs1288520, rs1288521, rs533375 and rs584626) and HDL-C (rs1288521, rs584626 and rs585131) in the non-HApoB100 individuals were different between the genotypes ($P < 0.05$-0.001).

After adjusting gender, age, race/ethnicity, BMI, tobacco smoking and alcohol consumption, logistic regression analysis demonstrated that the SNPs of rs584626 and rs540796 were associated with HTC; rs320018 and rs533375 were associated with HTG; rs320017 and rs540796 were associated with HLDL-C; rs320017, rs320018, rs1288521 and rs533375 were associated with HApoB100 ($P < 0.05$; **Table 2**).

Linkage disequilibrium (LD) analyses

The LD analyses of the detected SNPs with their pair-wise *D'*value and r^2 values revealed 9 gene-gene interaction models with $r^2 > 0.8$ ($r^2 = 0.90$ for rs320017 and rs320018; $r^2 = 0.95$ for rs320018 and rs320019; $r^2 = 0.88$ for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AGBL4 rs320018 A>G
AGBL4 rs320019 G>A
LRP8 rs6694764 G>A
LRP8 rs1288519 A>C
LRP8 rs872315 G>A
LRP8 rs1288520 C>T
LRP8 rs1288521 C>T
TC
HDL-C
LDL-C
ApoA1
ApoB100
ApoA1/ApoB100
HTC
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TG
AA
AG/GG
GG
AG/AA
AC/CC
CC
CT/TT

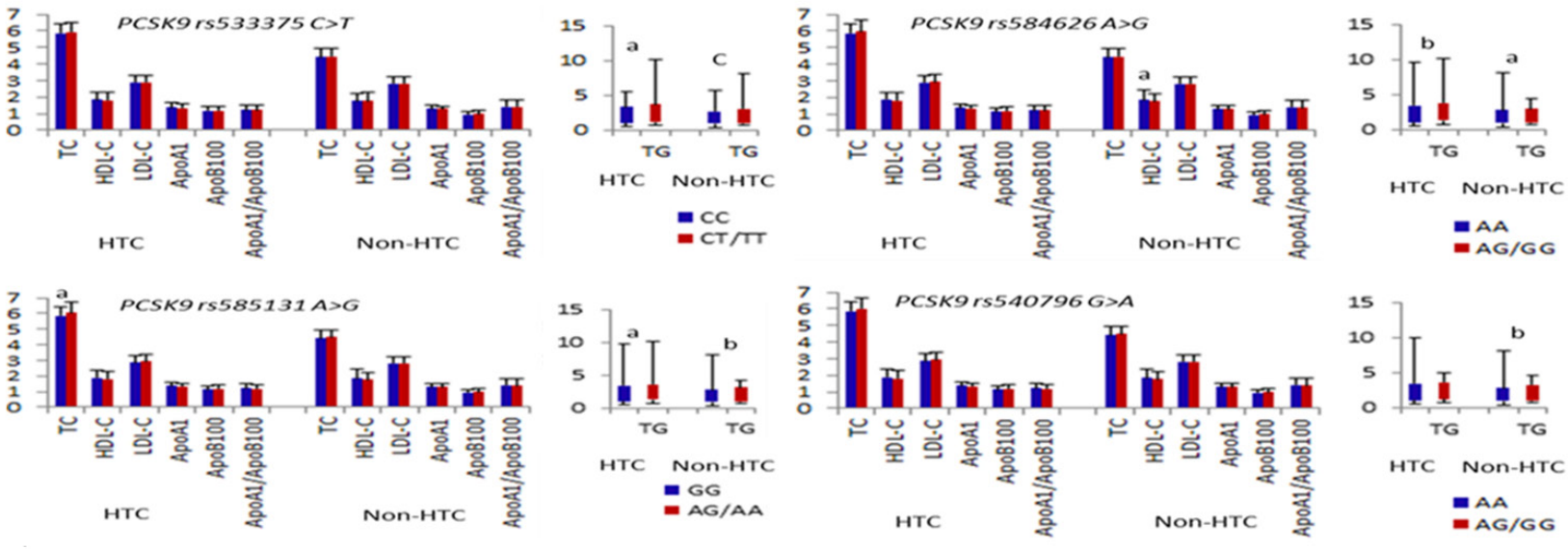
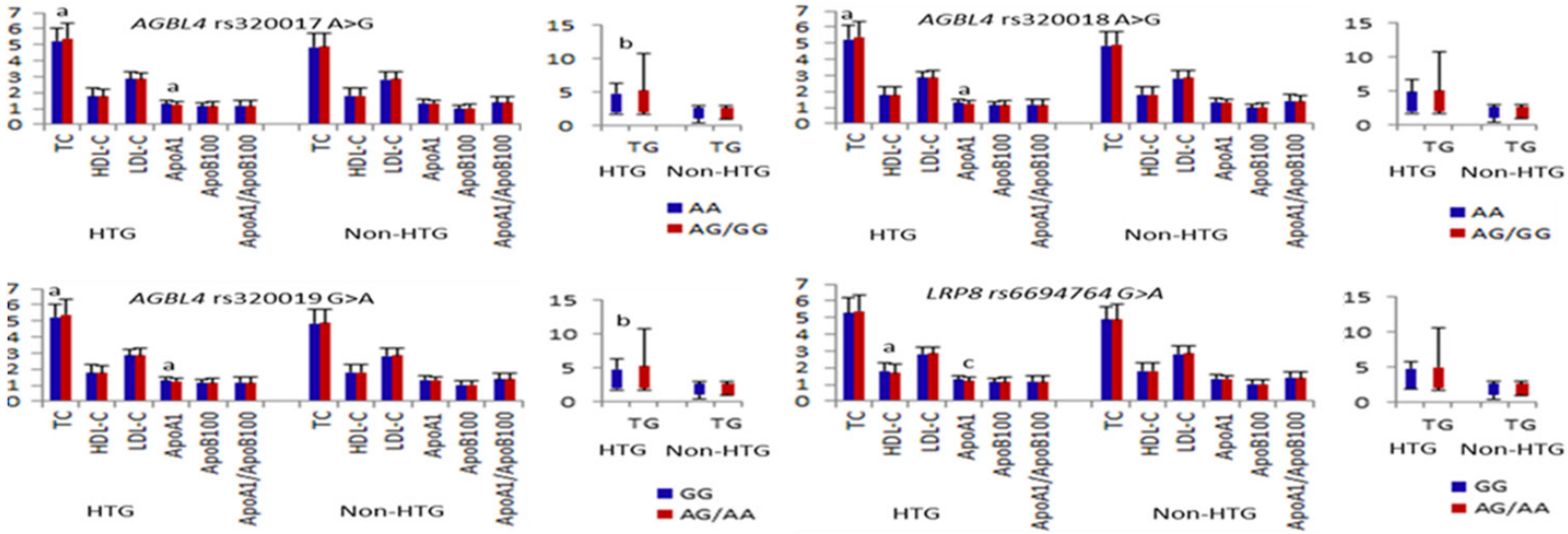


Figure 1. *AGBL4*, *LRP8* and *PCSK9* variants on high total cholesterol (HTC); [a]$P < 0.05$; [b]$P < 0.01$; [c]$P < 0.001$ *vs.* control.



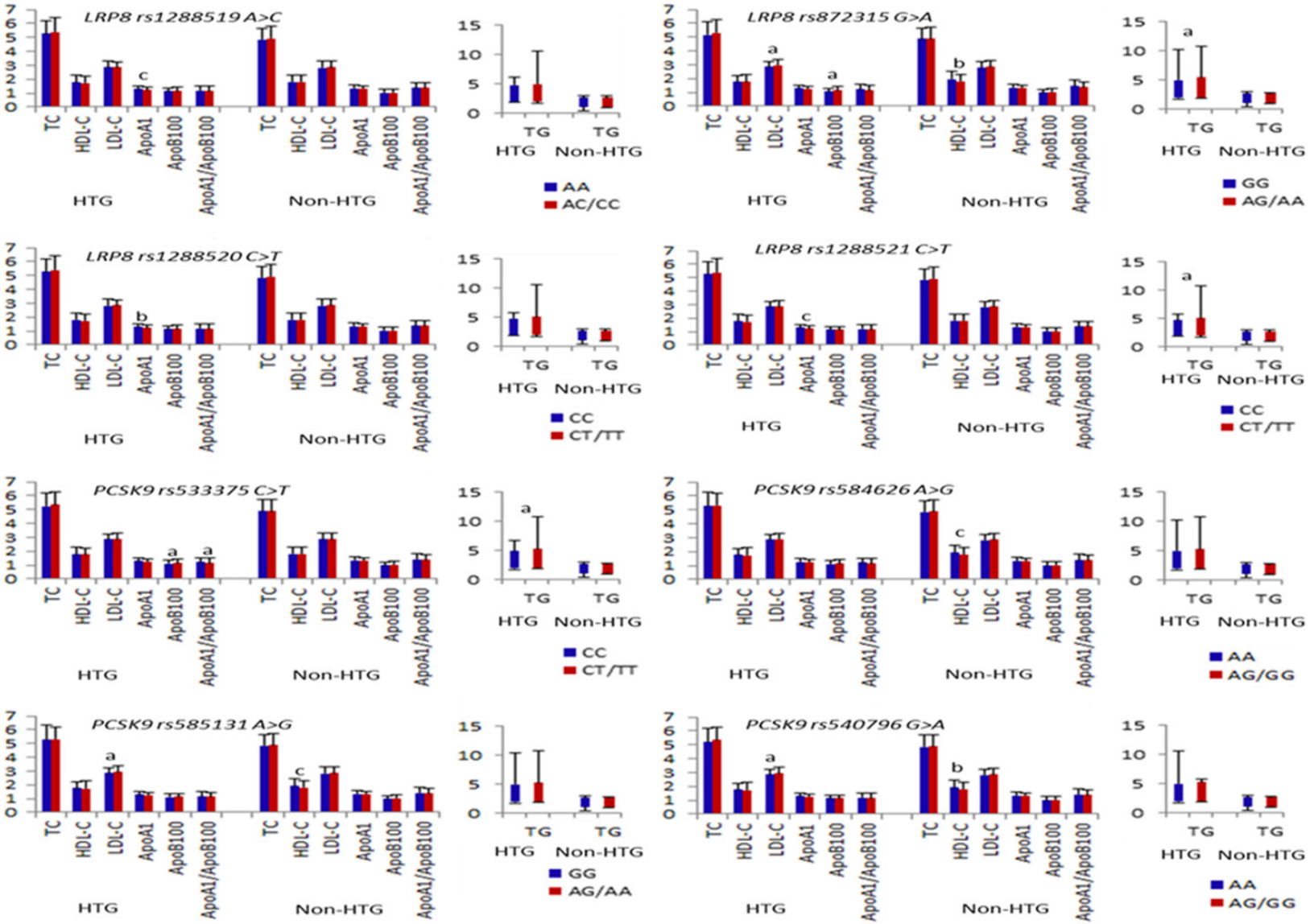
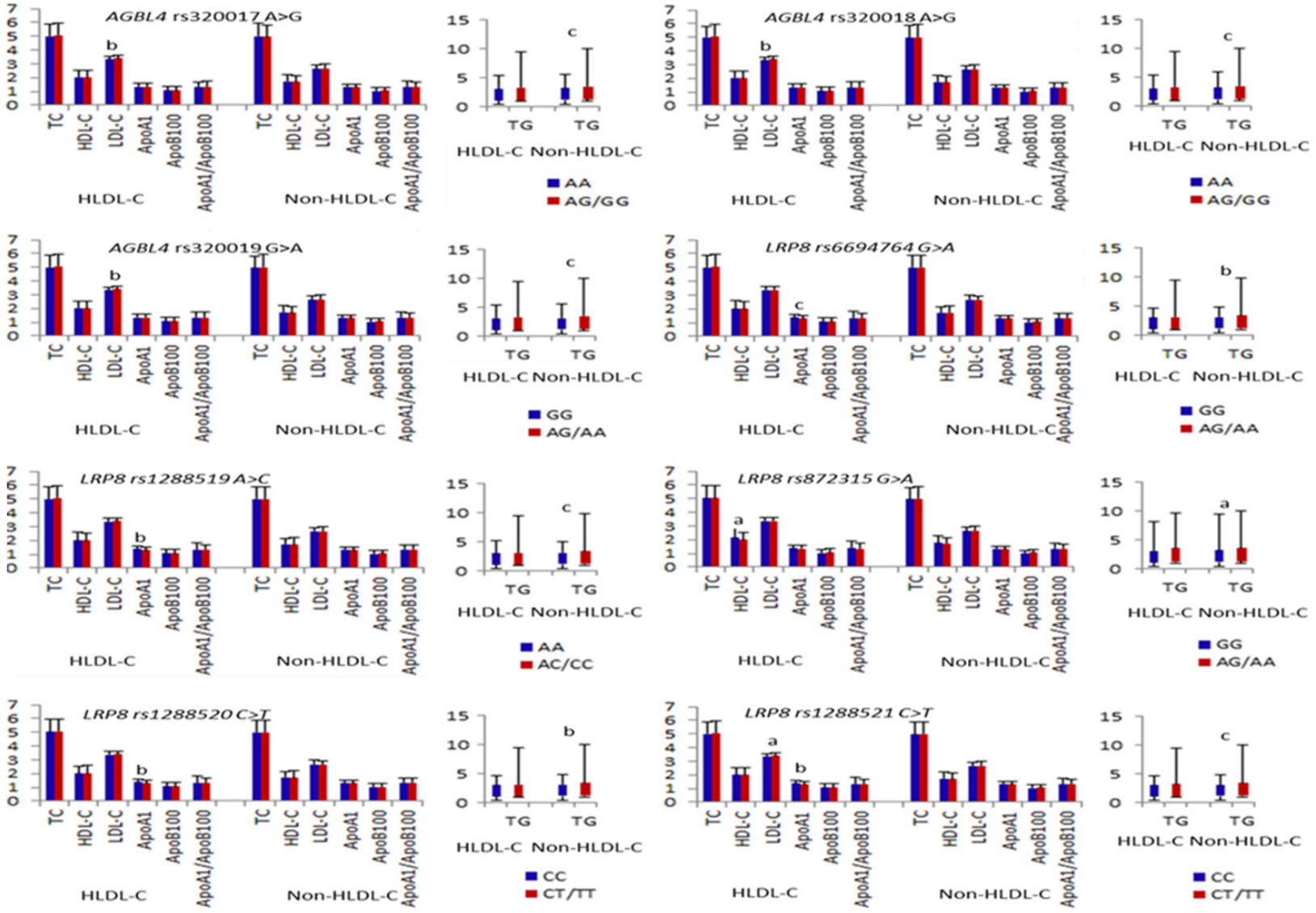


Figure 2. *AGBL4*, *LRP8* and *PCSK9* variants on high triglyceride (HTG); [a]$P < 0.05$; [b]$P < 0.01$; [c]$P < 0.001$ *vs.* control.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AGBL4 rs320018 A>G
AGBL4 rs320019 G>A
LRP8 rs6694764 G>A
LRP8 rs1288519 A>C
LRP8 rs872315 G>A
LRP8 rs1288520 C>T
LRP8 rs1288521 C>T
TC
HDL-C
LDL-C
ApoA1
ApoB100
ApoA1/ApoB100
HLDL-C
Non-HLDL-C
TG
AA
AG/GG
GG
AG/AA
AC/CC
CC
CT/TT

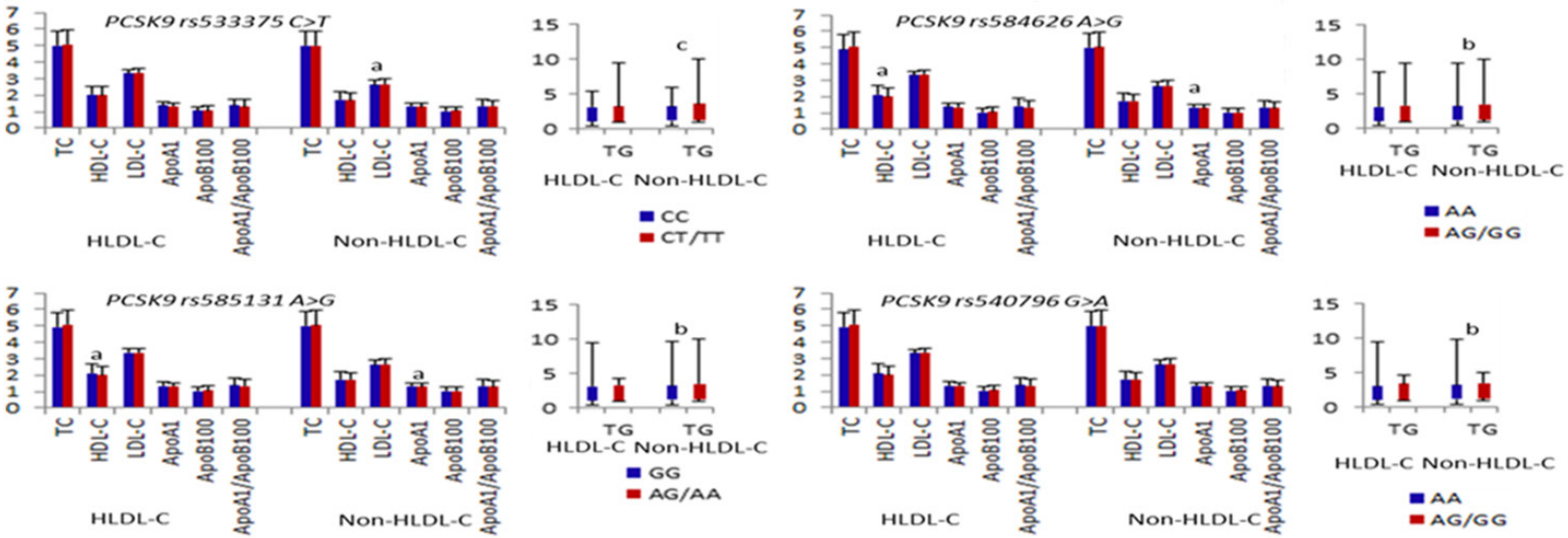
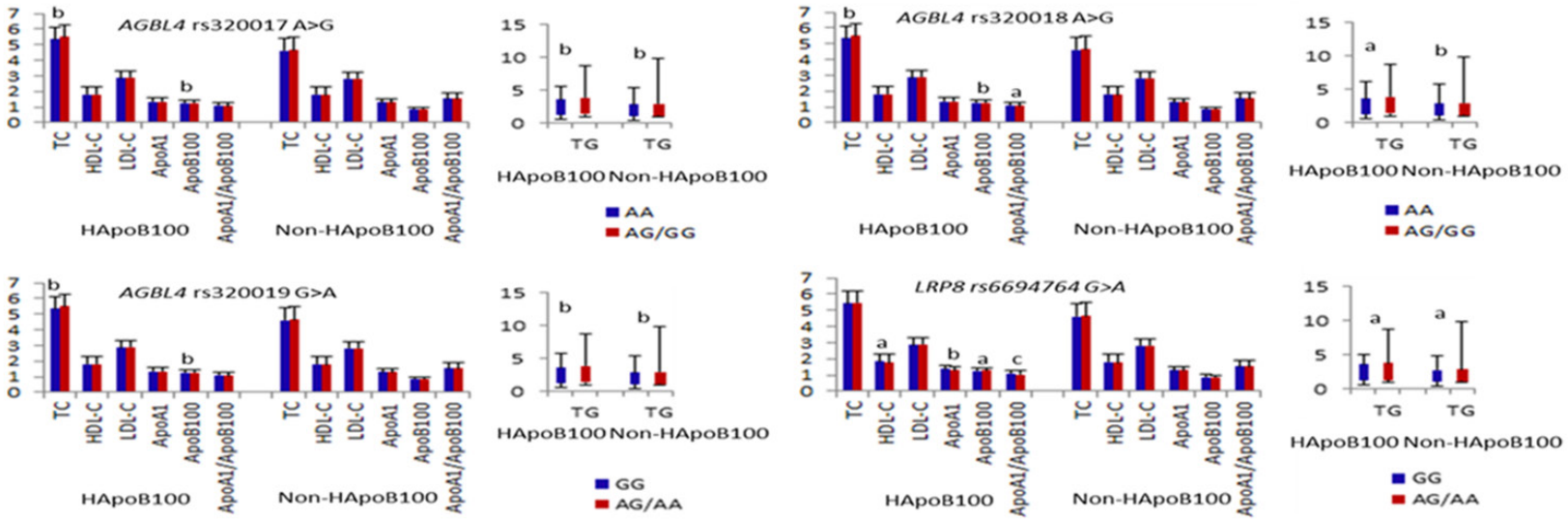


Figure 3. *AGBL4*, *LRP8* and *PCSK9* variants on high low-density lipoprotein cholesterol (HLDL-C); [a]$P < 0.05$; [b]$P < 0.01$; [c]$P < 0.001$ *vs.* control.



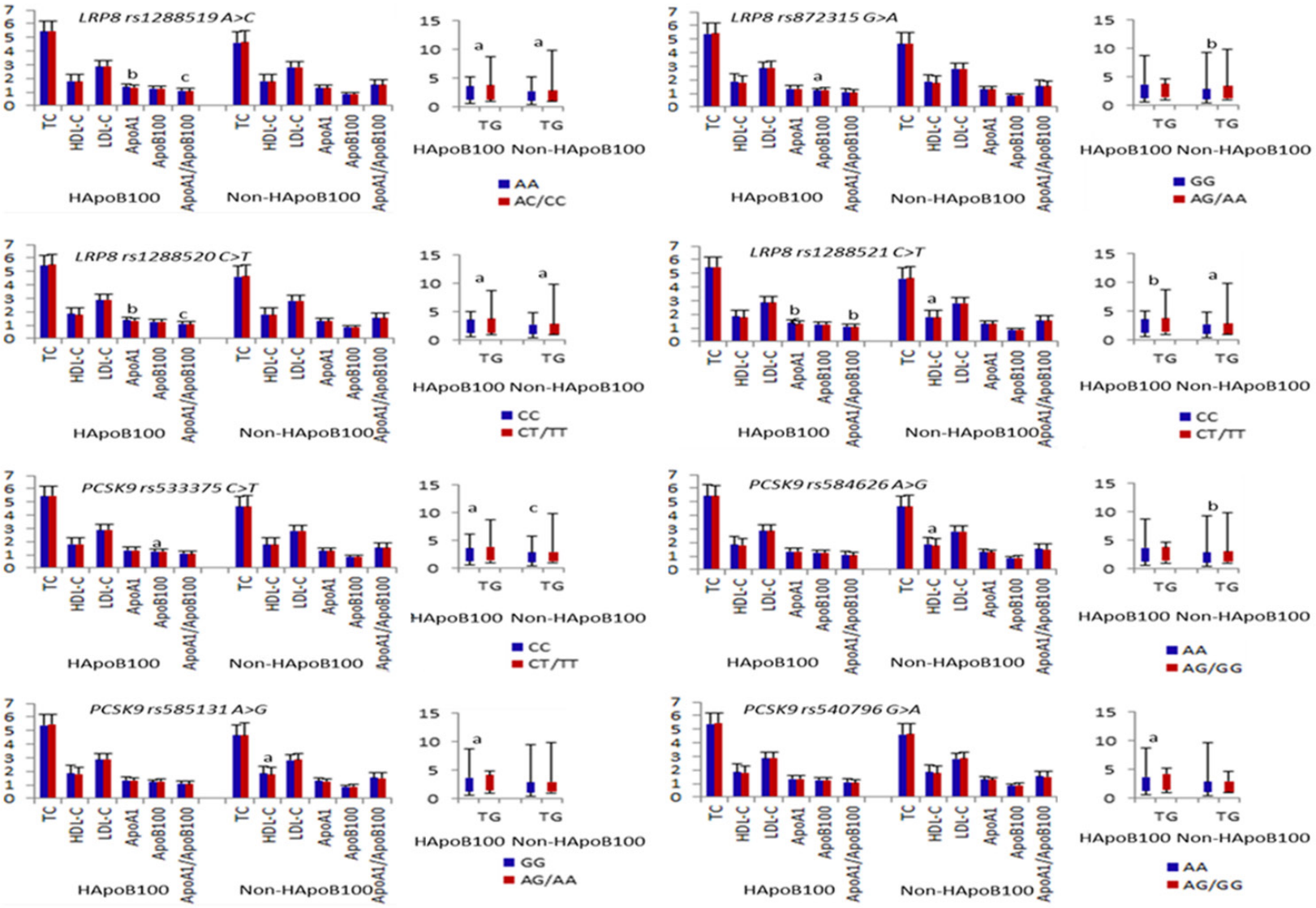


Figure 4. *AGBL4*, *LRP8* and *PCSK9* variants on high apolipoprotein B100 (HApoB100); [a]$P < 0.05$; [b]$P < 0.01$; [c]$P < 0.001$ *vs.* control.

Table 3. *AGBL4*, *LRP8* and *PCSK9* interactions on dyslipidemia (with *P*-value < 0.05)

Haplotype	Cases, n (feq)	Control, n (feq)	X^2	*P* Fisher's	*P* Pearson's	OR (95% CI)	Dyslipidemia
G-G-A-A-C-G-T-T-C-A-A-G	105.04 (0.051)	199.22 (0.065)	4.001	0.045537	0.045500	0.781 [0.612~0.996]	HTC
A-A-G-G-A-G-C-C-C-A-A-G	745.59 (0.504)	2163.95 (0.597)	37.36	1.06E-09	1.01E-09	0.685 [0.607~0.774]	HTG
G-G-A-A-C-G-T-T-T-A-A-G	175.54 (0.119)	287.97 (0.079)	19.504	1.03E-05	1.02E-05	1.559 [1.279~1.901]	HTG
A-A-G-A-C-G-T-T-C-A-A-G	216.00 (0.160)	510.65 (0.136)	4.908	0.02678	0.026757	1.216 [1.023~1.445]	HLDL-C
A-A-G-G-A-G-C-C-C-A-A-G	732.98 (0.545)	2176.57 (0.579)	4.849	0.027711	0.027688	0.869 [0.766~0.985]	HLDL-C
G-G-A-A-C-G-T-T-T-G-G-A	37.01 (0.027)	59.68 (0.016)	7.197	0.007326	0.007319	1.752 [1.157~2.653]	HLDL-C
A-A-G-A-C-G-T-C-C-A-A-G	67.02 (0.028)	113.94 (0.041)	6.23	0.01259	0.012579	0.678 [0.499~0.922]	HApoB100
Rare Hap (frequency < 3%) have been dropped							

Order: *AGBL4* rs320017, *AGBL4* rs320018, *AGBL4* rs320019, *LRP8* rs6694764, *LRP8* rs1288519, *LRP8* rs872315, *LRP8*rs1288520, *LRP8* rs1288521, *PCSK9* rs533375, *PCSK9* rs584626, *PCSK9* rs585131, *PCSK9* rs540796. HTC, hypercholesterolemia; HTG, hypertriglyceridemia; HLDL-C, hyper low-density lipoprotein cholesterol; HApoB100, hyper apolipoprotein (Apo) B100.

Table 4. Best inter-locus interaction models identified by the generalized multifactor dimensionality reduction method

Locus no	Best combination for HTC	Cross-validation consistency	Testing accuracy	*P*-value
2	rs320019 rs540796	7/10	0.5111	0.8281
3	rs320019 rs1288521 rs540796	10/10	0.5322	0.0547
Locus no	Best combination for HTG	Cross-validation consistency	Testing accuracy	*P*-value
2	rs320019 rs540796	6/10	0.5449	0.0010
3	rs320017 rs320019 rs540796	3/10	0.5392	0.0107
Locus no	Best combination for HLDL-C	Cross-validation consistency	Testing accuracy	*P*-value
2	rs1288520 rs540796	7/10	0.5273	0.0547
3	rs320017 rs1288520 rs540796	4/10	0.5235	0.0547
Locus no	Best combination for HApoB100	Cross-validation consistency	Testing accuracy	*P*-value
2	rs320019 rs540796	8/10	0.5208	0.0107
3	rs1288521 rs320019 rs540796	8/10	0.5310	0.0107

HTC, hypercholesterolemia; HTG, hypertriglyceridemia; HLDL-C, hyper low-density lipoprotein cholesterol; HApoB100, hyper apolipoprotein (Apo) B100.

rs320017 and rs320019; r^2 = 0.94 for rs6694764 and rs1288519; r^2 = 0.93 for rs6694764 and rs1288520; r^2 = 0.93 for rs1288519 and rs1288520; r^2 = 0.83 for rs1288519 and rs1288521; r^2 = 0.82 for rs1288520 and rs1288521; and r^2 = 0.83 for rs584626 and rs585131), 6 gene-gene interaction models with r^2 between 0.5 to 0.8 and another 51 gene-gene interaction models with r^2 < 0.5 in this study.

Haplotype frequencies

The haplotype frequencies are list in **Table 3**. The commonest haplotype was A-A-G-G-A-G-C-C-C-A-A-G (in order of *AGBL4* rs320017, *AGBL4* rs320018, *AGBL4* rs320019, *LRP8* rs6694764, *LRP8* rs1288519, *LRP8* rs872315, *LRP8* rs1288520, *LRP8* rs1288521, *PCSK9* rs533375, *PCSK9* rs584626, *PCSK9* rs585131 and *PCSK9* rs540796; > 50% of the samples; HTC), A-A-G-G-A-G-C-C-C-A-A-G (> 50% of the samples; HTG), A-A-G-G-A-G-C-C-C-A-A-G (> 50% of the samples; HLDL-C) and A-A-G-G-A-G-C-C-C-A-A-G (> 50% of the samples; HApoB100). After dropping rare haplotypes (frequency < 3%), the haplotype frequencies of the G-G-A-A-C-G-T-T-C-A-A-G were different between the HTC and non-HTC individuals. The G-G-A-A-C-G-T-T-C-A-A-G was associated with a reduced risk of HTC (OR: 0.781, 95% CI: 0.612-0.996, *P* < 0.05). The haplotype frequencies of the A-A-G-G-A-G-C-C-C-A-A-G and G-G-A-A-C-G-T-T-T-A-A-G were different between the HTG and non-HTG individuals. The A-A-G-G-A-G-C-C-C-A-A-G was associated with a reduced risk of HTG (OR: 0.685, 95% CI: 0.607-0.774, *P* < 0.001), and the G-G-A-A-C-G-T-T-T-A-A-G was associated with increased risk of HTG (OR: 1.559, 95% CI: 1.279-1.901, *P* < 0.001). The A-A-G-A-C-G-T-T-C-A-A-G, A-A-G-G-A-G-C-C-C-A-A-G and G-G-A-A-C-G-T-T-T-G-G-A were different between the HLDL-C and non-HLDL-C individuals. The A-A-G-G-A-G-C-C-C-A-A-G was associated with

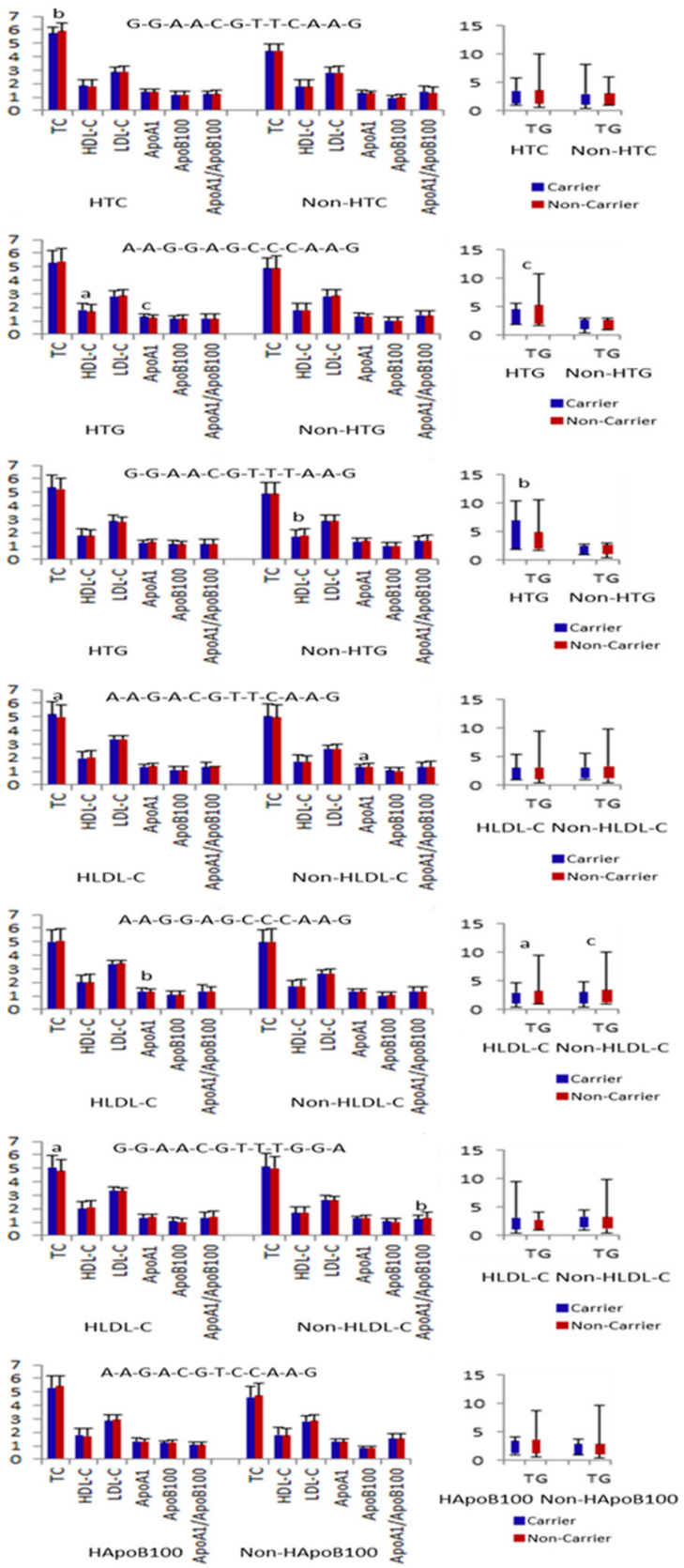


Figure 5. *AGBL4*, *PRL8* and *PCSK9* interactions on dyslipidemia; [a]*P* < 0.05; [b]*P* < 0.01; [c]*P* < 0.001 *vs.* control.

a reduced risk of HLDL-C (OR: 0.869, 95% CI: 0.766-0.985, *P* < 0.05), and A-A-G-A-C-G-T-T-C-A-A-G (OR: 1.216, 95% CI: 1.023-1.445, *P* < 0.05) and G-G-A-A-C-G-T-T-T-G-G-A (OR: 1.752, 95% CI: 1.157-2.653, *P* < 0.01) were associated with increased risk of HLDL-C. The A-A-G-A-C-G-T-C-C-A-A-G was different between the HApoB100 and non-HApoB100 individuals. The A-A-G-A-C-G-T-C-C-A-A-G (OR: 0.678, 95% CI: 0.499-0.922, *P* < 0.05) was associated with a reduced risk of HApoB100.

Gene-gene interaction models analyses

Table 4 shows the impacts of combination among the *AGBL4*, *PRL8* and *PCSK9* variants, which were analyzed by GMDR. The two- and three-locus models showed a significant association with the risk of HTG and HApoB100 (*P* < 0.05-0.005) and there is no significant statistical association two-and three-locus models with the risk of HTC and HLDL-C (*P* > 0.05). The two-locus model was chosen as the best one, owing to the fact of having the highest level of testing accuracy (54.49% for HTG and 52.08% for HapoB100) and good cross-validation consistency (6/10 for HTG and 8/10 for HapoB100). The three-locus model was chosen as the best one, owing to the fact of having the highest level of testing accuracy (53.92% for HTG and 53.10% for HapoB100) and good cross-validation consistency (3/10 for HTG and 8/10 for HapoB100).

AGBL4, LRP8 and PCSK9 interactions on dyslipidemia

The correlation of the haplotypes of the *AGBL4*, *PRL8* and *PCSK9* and serum lipid phenotypes is shown in **Figure 5**. Rare Hap (frequency < 3%) has been dropped.

The carriers of G-G-A-A-C-G-T-T-C-A-A-G had lower TC level in HTC individuals than the non-carriers of G-G-A-A-C-G-T-T-C-A-A-G ($P < 0.01$). There were no differences in serum lipid phenotypes between the carriers and non-carriers of G-G-A-A-C-G-T-T-C-A-A-G in non-HTC individuals. The A-A-G-G-A-G-C-C-C-A-A-G carriers had higher serum HDL-C and ApoA1 and lower TG levels in HTG individuals than the A-A-G-G-A-G-C-C-C-A-A-G non-carriers ($P < 0.05$-0.001). There were no differences in serum lipid phenotypes between the carriers and non-carriers of A-A-G-G-A-G-C-C-C-A-A-G in non-HTG individuals. The G-G-A-A-C-G-T-T-T-A-A-G carriers had higher serum TG levels in HTG individuals and lower serum HDL-C levels in non-HTG individuals than the G-G-A-A-C-G-T-T-T-A-A-G non-carriers ($P < 0.01$ for each). The A-A-G-A-C-G-T-T-C-A-A-G carriers had higher serum TC levels in HTG individuals and lower serum ApoA1 levels in non-HTG individuals than the A-A-G-A-C-G-T-T-C-A-A-G non-carriers ($P < 0.05$ for each). The A-A-G-G-A-G-C-C-C-A-A-G had lower serum TG and higher ApoA1 levels in HLDL-C individuals and higher ApoA1 levels in non-HTG individuals than the A-A-G-G-A-G-C-C-C-A-A-G non-carriers ($P < 0.05$-0.001). The G-G-A-A-C-G-T-T-T-G-G-A carriers had higher serum TC levels in HTG individuals and lower ratio of ApoA1 to ApoB100 in non-HTG individuals than the G-G-A-A-C-G-T-T-T-G-G-A non-carriers ($P < 0.05$-0.01). There were no differences in serum lipid phenotypes between the carriers and non-carriers of A-A-G-A-C-G-T-C-C-A-A-G in both HApoB100 and non-HApoB100 individuals ($P > 0.05$ for all).

Discussion

In the present study, we illuminated that (*i*) the *AGBL4*, *PRL8* and *PCSK9* variants associate with dyslipidemia, comprising HTC, HTG, HLDL-C and HApoB100; (*ii*) their haplotypes of the detected variants involved in mechanisms of dyslipidemia; and (*iii*) the possible gene-gene interaction models among these variants are useful for identifying more precise and distinct susceptible signals of dyslipidemia, comparing with single-locus test. This is the first study to comprehensively explore the inter-locus interactions among the *AGBL4*, *PRL8* and *PCSK9* genetic variants on dyslipidemia.

There is almost universal agreement that patients with established ASCVD should receive cholesterol-lowering drugs. Ideally, LDL-C and non-HDL-C should be reduced to very low level (at least a therapeutic target level). Yet, many patients cannot achieve these very low levels, even with high doses of powerful statins [30]. A newer class of drugs, called PCSK9 inhibitors, powerfully reduces LDL-C; PCSK9 inhibitors are currently being tested to determine how much additional risk reduction occurs when combined with high doses of statins [31]. Not only an emerging therapeutic target, *PCSK9* but also is the third causal gene of familial hypercholesterolemia [32]. The gain-of-function variants, comprising E32K in *PCSK9* [33] were found commonly in Japanese familial hypercholesterolemia. Consequently, we investigated *PCSK9*, *AGBL4* and *LRP8*, which their cytogenetic locations are very closely of human chromosome 1 [34].

This is the first report that the *AGBL4* variants on serum lipid levels. A previous study reported that the *LRP8* R952Q (rs5174) was associated with TG levels in 358 Gene Quest Caucasian probands, and this finding was replicated in one other independent population of 134 patients with early-onset myocardial infarction [35]. Moreover, several papers reported that (*i*) the *PCSK9* E670G variant in a European population was associated with increased LDL-C in men but not in women; (*ii*) the L46 allele not only decreased LDL-C but also protected against myocardial infarction in the Italian populations [36]; (*iii*) the *PCSK9* rs11591147 was associated with low cholesterol levels in Scotland (Glasgow), Ireland (Cork), and the Netherlands (Leiden) [37]; and (iv) one person who was homozygous for *PCSK9* R46L had LDL-C levels of 11 mg/dL. In one family, 6 out of 8 members carrying the R46L variant had LDL-C levels below the lower 10% percentile of LDL-C among all study participants in the Strong Heart Family Study (SHFS) [38]. In current study, we demonstrated that the *PCSK9* rs584626 G-allele carries increased risk of HTC and *PCSK9* rs540796 A-allele carries protected against HTC; *AGBL4* rs320018 G-allele carries protected against HTG and *PCSK9* rs533375 T-allele carries increased risk of HTG; *AGBL4* rs320017 G- and *PCSK9* rs540796 A-allele carries increased risk of HLDL-C; *AGBL4* rs320017 G- and *PCSK9* rs533375 T-allele carries increased risk of HApoB100 and *AGBL4* rs320018 G- and *LRP8* rs1288521 T-allele carries protected against HApoB100.

Dyslipidemia is a complex disease; single gene is difficult to perfectly explain the mechanism. The one gene significantly results may be the consequence of linkage disequilibrium with other genes. In this study, we noticed that the *AGBL4*, *LRP8* and *PCSK9* interaction model of G-G-A-A-C-G-T-T-C-A-A-G, the *PCSK9* rs58-4626 G-allele non-carries, protected against HTC; A-A-G-G-A-G-C-C-C-A-A-G, the *PCSK9* rs533375 T-allele non-carries, was associated with reduced risk of HTC, and G-G-A-A-C-G-T-T-T-A-A-G, the *PCSK9* rs533375 T-allele carries, was associated with increased risk of HTG. G-G-A-A-C-G-T-T-T-G-G-A, the *AGBL4* rs32-0017 G- and *PCSK9* rs540796 A-allele carries was associated increased risk of HLDL-C; and A-A-G-A-C-G-T-C-C-A-A-G, the *AGBL4* rs320017 G- and *PCSK9* rs533375 T-allele non-carries protected against HApoB100.

On GMDR analysis, an inter-locus interaction among the *AGBL4*, *LRP8* and *PCSK9* on dyslipidemia was found in present study. The interactions of *AGBL4* rs320019-*PCSK9* rs540796 and *AGBL4* rs320017-*AGBL4* rs320019-*PCSK9* rs540796 were associated with the risk of HTG, and *AGBL4* rs320019-*PCSK9* rs540796 and *LRP8* rs1288521-*AGBL4* rs320019-*PCSK9* rs540796 were associated with the risk of HApoB100. In multi-locus (GMDR) analyses, a significant association with HTG and HApoB100 was found in two-to three-locus models. These findings indicate that a potential gene-gene interaction might exist among the *AGBL4*, *LRP8* and *PCSK9* variants. Unfortunately, no previous study has investigated the inter-locus interaction among *AGBL4*, *LRP8* and *PCSK9* variants, and therefore we cannot make comparisons with our results. Although, a statistically significant haplotype was noted in this study, the biological mechanism underlying these genes and their interactions are still yet to be defined.

There are several potential limitations in our study. Firstly, the number of participants available for minor allele frequency (MAF) of some variants was not high enough to calculate a strong power as compared with many previous GWASs and replication studies. Secondly, we were unable to alleviate the effect of diet, such as rans, saturated, polyunsaturated (n-3 and n-6), and monounsaturated fatty acids, during the statistical analysis. Thirdly, the relevance of this finding has to be defined in further high caliber of studies including incorporating the genetic information of the *AGBL4*, *LRP8* and *PCSK9* variants and their interactions *in vitro* and *in vivo* functional studies to confirm the impact of a variant on a molecular level. What's more, discussion of race and ethnicity in medicine must rigorously avoid polarization and the further perpetuation of disparate health care.

Conclusions

Our study confirmed that the genetic variants are replicable in the Chinese HTC/HTG/HLDL-C/HApoB100 and non-dyslipidemia populations. Haplotypes could explain much more serum lipid variation than any single-variant alone. Differences in serum lipid phenotypes among these populations might partially attribute to *AGBL4*, *LRP8* and *PCSK9* variants and their haplotypes. However, further functional studies of these genes are still required to clarify which variants are functional and how these genes actually affect serum lipid phenotypes.

Taken all of facts into consideration, it is possible that the significant variants identified in the *AGBL4*, *LRP8* and *PCSK9* region might be in high linkage disequilibrium with some of the functional variants in other genes, which is known to affect the risk of dyslipidemia. Thus, an in-depth study of the biological actions of these genes is crucial to clarify which variants are functional and how these genes actually affect the risk of dyslipidemia. It is expected that the physiological function of *AGBL4*, *LRP8* and *PCSK9* will be elucidated in a not too distant future.

Acknowledgements

This study was supported by the National Natural Science Foundation of China (No: 8116-0111) and the Innovation Project of Guangxi Graduate Education. The authors are grateful to the participants and their families, as well as to the First Affiliated Hospital of Guangxi Medical University.

Disclosure of conflict of interest

None.

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