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Original article

Association of *CELSR2*, *APOB100*, *ABCG5/8*, *LDLR*, and *APOE* polymorphisms and their genetic risks with lipids among the Thai subjects

Manit Nuinoon [a,e], Wutthichai Saiphak [a], Nantiya Nawaka [a], Chutima Rattanawan [a,e], Burabha Pussadhamma [b,c], Nutjaree Jeenduang [a,d,*]

[a] School of Allied Health Sciences, Walailak University, Nakhon Si Thammarat, Thailand
[b] Department of Internal Medicine, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand
[c] Queen Sirikit Heart Center of the Northeast, Khon Kaen University, Khon Kaen, Thailand
[d] Food Technology and Innovation Research Center of Excellence, Walailak University, Nakhon Si Thammarat, Thailand
[e] Hematology and Transfusion Science Research Center, Walailak University, Nakhon Si Thammarat, Thailand

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ABSTRACT

Background: Hypercholesterolemia is a common cardiovascular risk factor. The aim of this study was to investigate the association of *CELSR2* (rs629301), *APOB100* (rs1367117), *ABCG5/8* (rs6544713), *LDLR* (rs6511720), and *APOE* (rs429358, rs7412) polymorphisms, and their genetic risk scores with lipids among Thai subjects.

Methods: A total of 459 study subjects (184 males, and 275 females) were enrolled. Blood pressure, serum lipids, and fasting blood sugar were measured. *CELSR2* (rs629301), *APOB100* (rs1367117), *ABCG5/8* (rs6544713), and *LDLR* (rs6511720) polymorphisms were analyzed using PCR-HRM. *APOE* (rs429358, rs7412) polymorphism was analyzed using PCR-RFLP.

Results: Total cholesterol (TC) levels were significantly higher in *APOB100* AA genotype compared with GG, or AA + AG genotypes in total subjects. In addition, significantly higher concentrations of TC and low density lipoprotein cholesterol (LDL-C) were observed in *APOE4* carriers compared to *APOE2* carriers in total subjects, males, and females. The significantly higher concentrations of TC were observed in *APOE4* carriers compared to *APOE3* carriers in females. Moreover, the concentrations of TC, and LDL-C were significantly increased with genetic risk scores of *APOB100*, and *APOE* polymorphisms in total subjects, and females. There was no association between *CELSR2* (rs629301), *ABCG5/8* (rs6544713), and *LDLR* (rs6511720) polymorphisms and serum lipids.

Conclusion: *APOB100* (rs1367117), and *APOE* (rs429358, rs7412) but not *CELSR2* (rs629301), *ABCG5/8* (rs6544713), and *LDLR* (rs6511720) polymorphisms were associated with serum lipids. The cumulative risk alleles of *APOB100* (rs1367117), and *APOE* (rs429358, rs7412) polymorphisms could enhance the elevated concentrations of TC, and LDL-C, and they may be used to predict severity of hypercholesterolemia among Thai subjects.

1. Introduction

Hypercholesterolemia is a common cardiovascular risk factor (Kreisberg et al. 2002), and it results from several risk factors, including genetic, and environmental factors (Ruixing et al. 2007), as well as, gene-environment interaction (Yin et al. 2011). Several environmental factors e.g. dietary, alcohol intake, obesity, smoking, and lack of exercise were found to be associated with hypercholesterolemia (Heller et al. 1993). In addition, genetic fac-

* Corresponding author at: School of Allied Health Sciences, Walailak University, Nakhon Si Thammarat, Thailand.
E-mail addresses: manit.nu@wu.ac.th (M. Nuinoon), wutthichai.sa@mail.wu.ac.th (W. Saiphak), nantiya.na@mail.wu.ac.th (N. Nawaka), chutima.rt@wu.ac.th (C. Rattanawan), burapu@kku.ac.th (B. Pussadhamma), nutjaree.je@wu.ac.th (N. Jeenduang).

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tors have been reported to influence serum lipids for 40–70 % of interindividual variation (Heller et al. 1993).

The genome-wide association studies (GWAS) by the Global Lipid Genetic Consortium (GLGC) demonstrated that 95 loci influenced LDL-C levels (Teslovich et al. 2010). Among these loci, 6 single nucleotide polymorphisms (SNPs) including *CELSR2* (rs629301), *APOB100* (rs1367117), *ABCG5/8* (rs6544713), *LDLR* (rs6511720), and *APOE* (rs429358, rs7412) which had a strong relationship with increased LDL-C levels were able to discriminate the mutation-positive familial hypercholesterolemia (FH/M +), and mutation-negative familial hypercholesterolemia (FH/M-), and diagnose polygenic hypercholesterolemia (Talmud et al. 2013, Futema et al. 2015). FH is an autosomal dominant disease that is characterized by severe hypercholesterolemia (Alharbi et al. 2017). Nevertheless, these 6 SNPs could not be used to distinguish the FH/M-, and FH/M+, but they could be used as the genetic marker for predicting the severity of hypercholesterolemia in other studies (Ghaleb et al. 2018, Rieck et al. 2020). Another study also demonstrated that the frequency of angiotensin-converting enzyme (ACE) insertion/deletion (I/D) polymorphism was not significantly different between FH, and control in the Saudi population (Alharbi et al. 2013).

CELSR2, APOB100, ABCG5/8, LDLR, and *APOE* genes encoded the protein that played an important role in the lipoprotein metabolism. Cadherin EGF LAG Seven-Pass G-Type Receptor 2 (*CELSR2*) was located on chromosome 1p13 (Kathiresan et al. 2009a). CELSR2 played a role in cell adhesion, and receptor-ligand interactions (Kathiresan et al. 2009a). It was in strong linkage disequilibrium with *PSRC1*, and *SORT1* genes (Musunuru et al. 2010). Apolipoprotein B100 (*APOB100*) was located on chromosome 2p24.1 (Law et al. 1985). It was the main apolipoprotein on low-density lipoprotein (LDL), and chylomicrons (Lu et al. 2001). ATP-binding cassette half-transporters G5 (*ABCG5*), and G8 (*ABCG8*) were located on chromosome 2p21 (Berge et al. 2000, Lu et al. 2001). It promoted the secretion of neutral sterols into bile (Berge et al. 2000, Lu et al. 2001). Low-density lipoprotein receptor (*LDLR*) was located on chromosome 19 (19p13.1–13.3) (Goldstein et al. 2009). It played a role in binding to the apoB100 or apoE on LDL, and uptake LDL and internalized into the hepatocytes via receptor-mediated endocytosis (Goldstein et al. 2009). Finally, apolipoprotein E (*APOE*) was located on chromosome 19q13.32 (Mahley et al. 2000). APOE acted as the ligand for LDLR, and it regulated cholesterol homeostasis by the uptake of VLDL, IDL, and chylomicron remnants into the hepatocytes (Mahley et al. 2000).

To our knowledge, the association study of *CELSR2* (rs629301), *APOB100* (rs1367117), *ABCG5/8* (rs6544713), and *LDLR* (rs6511720) polymorphisms with serum lipids among Thai subjects has not yet been elucidated. Although *APOE* (rs429358, rs7412) polymorphism was associated with serum lipids among the Thai population (Wanmasae et al. 2017, Srirojnopkun et al. 2018), the effect of genetic risk scores of these polymorphisms on serum lipids have not been reported. Consequently, we aimed to investigate the association of *CELSR2* (rs629301), *APOB100* (rs1367117), *ABCG5/8* (rs6544713), *LDLR* (rs6511720), and *APOE* (rs429358, rs7412), and their genetic risk scores with serum lipids among Thai population.

2. Methods

2.1. Subjects

A total of 459 unrelated healthy volunteers from Nakhon Si Thammarat province, Southern Thailand were enrolled in this study. Subjects with diabetes mellitus, liver, thyroid, or renal disease, pregnant women, and subjects who received lipid-lowering drugs were excluded from the study. Body mass index (BMI), systolic blood pressure (SBP), and diastolic blood pressure (DBP) were measured. The protocol of this study was approved by the ethics committee of Walailak University (WUEC-20–356-01). The study was conducted according to the Declaration of Helsinki guidelines. All subjects gave informed consent before participating to the study.

2.2. Laboratory analysis

After 12 h of fasting, blood was drawn from all subjects, separated serum, and plasma by centrifuging for 10 min at 3,000 rpm. Fasting blood sugar, triglyceride (TG), total cholesterol (TC), and high-density lipoprotein cholesterol (HDL-C) were determined using enzymatic assay by the Konelab analyzer (KONELAB 20, Tokyo, Japan). Friedewald formula was used to calculate low-density lipoprotein cholesterol (LDL-C) (Friedewald et al. 1972).

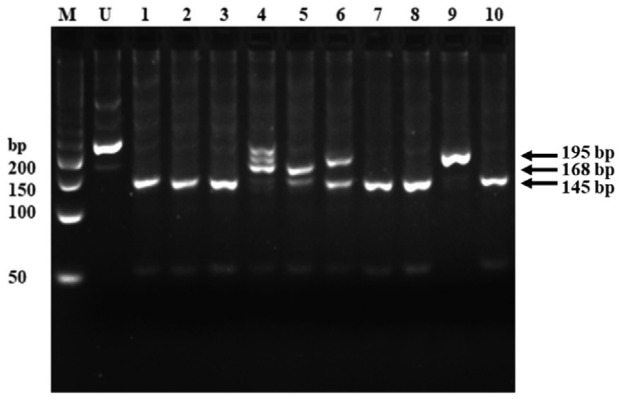
2.3. Genotyping of CELSR2 (rs629301), APOB100 (rs1367117), ABCG5/8 (rs6544713), and LDLR (rs6511720) polymorphisms

The Genomic DNA mini kit (Geneaid Biotech ltd., Taipei, Taiwan) was used to extract genomic DNA (gDNA) from blood leukocytes. Nanodrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA) was used to measure DNA concentration and its purity. The gDNA was kept at −80 °C until further analysis. Polymerase chain reaction and high resolution melting (PCR–HRM) analysis was performed to analyze *CELSR2* (rs629301), *APOB100* (rs1367117), *ABCG5/8* (rs6544713), and *LDLR* (rs6511720) polymorphisms by using the QuantStudio™ 3 Real-Time PCR System (Thermo Fisher Scientific, USA). The reaction mixture contained 1X HOT FIREPol® EvaGreen® HRM Mix (ROX) (Solid Biodyne, Tartu, Estonia), 500 nM of each primer, 20 ng of genomic DNA template, and deionized water to 20 μL. The primers for analysis of *CELSR2* (rs629301), *APOB100* (rs1367117), *ABCG5/8* (rs6544713), and *LDLR* (rs6511720) polymorphisms are presented in Table S1. The conditions of PCR-HRM for analysis of *CELSR2* (rs629301), *APOB100* (rs1367117), and *ABCG5/8* (rs6544713) polymorphisms are presented in Table S2. DNA sequencing of *CELSR2*, *APOB100*, *ABCG5/8*, and *LDLR* polymorphisms was performed to confirm the results.

2.4. Genotyping of APOE (rs429358, rs7412) polymorphism

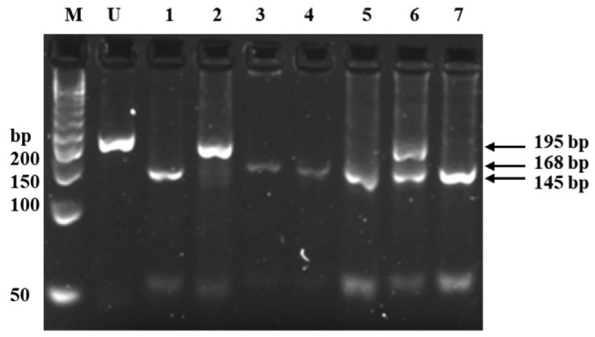
APOE (rs429358, rs7412) polymorphism was analyzed by the PCR-RFLP as described previously (Jeenduang et al. 2015). Briefly, the reaction mixture contained 0.2 μM of each primer, 100 ng genomic DNA, 200 μM dNTPs (NEB, USA), 1X buffer containing 1.5 mM $MgCl_2$, 0.625 U of *Taq* DNA polymerase (NEB, USA), 10 % dimethylsulphoxide (DMSO), and deionized water to 25 μL. The primers, and the PCR conditions for analysis of *APOE* (rs429358, rs7412) polymorphism are presented in Table S1, and Table S3, respectively. The PCR products were digested with *Afl*III and *Hae*II (NEB, USA) for 24 h at 37 °C. The undigested, and digested PCR products were run in 4 % agarose gel electrophoresis; at 100 V, for 60 min stained with SafeView™ and visualized using the Gel Doc XR gel documentation system (Bio-Rad Laboratories, Hercules, CA, USA). The undigested PCR products was 218 bp. After digestion, the fragments of sizes of E3, E2, and E4 alleles were 145, 168, and 195 bp, respectively (Fig. 1). DNA sequencing of *APOE* polymorphism was performed to confirm the results (Fig. 2).

Codon 112 (rs429358) Codon 158 (rs7412).



Lane M: 50 bp DNA ladder
Lane U: Undigested PCR products of 218 bp
Lane 1-3: E3E3 genotype; Lane 4: E2E4 genotype; Lane 5: E2E3 genotype
Lane 6: E3E4 genotype; Lane 7-8: E3E3 genotype; Lane 9: E4E4 genotype
Lane 10: E3E3 genotype

a)



Lane M: 50 bp DNA ladder
Lane U: Undigested PCR products of 218 bp
Lane 1: E3E3 genotype; Lane 2: E4E4 genotype; Lane 3-4: E2E2 genotype
Lane 5: E3E3 genotype; Lane 6: E3E4 genotype; Lane 7: E3E3 genotype

b)

Fig. 1. *APOE* genotyping by PCR-RFLP (a-b). 4 % agarose gel electrophoresis of *APOE* genotyping staining with SafeView™. After *Afl*III, and *Hae*II digestion, the PCR fragments of 195 bp, 168 bp, and 145 bp represented the E4, E2, and E3 alleles, respectively.

2.5. Statistical analyses

Data was shown as mean ± standard deviation (SD). Student *t*-test, or the Mann-Whitney *U* test was performed to analyze the differences between groups. One-way ANOVA, or Kruskal-Wallis test was performed for multiple comparisons of means among groups. The Chi-square test was performed for analyzing the Hardy–Weinberg equilibrium of *CELSR2* (rs629301), *APOB100* (rs1367117), *ABCG5/8* (rs6544713), *LDLR* (rs6511720), and *APOE* (rs429358, rs7412) polymorphisms. To calculate the genetic risk scores; for *CELSR2* (rs629301), *APOB100* (rs1367117), *ABCG5/8* (rs6544713), *LDLR* (rs6511720) genotyping; the model assigned 0, or + 1 for non risk allele, and risk allele, respectively [9–10]. Thus, the homozygous, and heterozygous for the risk allele, and homozygous for the non-risk allele had scores of 2, 1, and 0, respectively. For *APOE* genotyping, the model assigned −1, 0, or + 1 for E2, E3, or E4 alleles, respectively. The E2E2, E2E3, E2E4, E3E3, E3E4, and E4E4 genotypes then had scores of −2, −1, 0, 0, 1, and 2, respectively. The relationship between genetic risk scores, and serum lipids was evaluated by multivariate linear regression analysis. SPSS (SPSS Inc., Chicago, IL; Version 23) was used to analyze all data. A p-value<0.008 (p value = 0.05/6 SNPs) was statistically significant, following the Bonferroni correction.

3. Results

3.1. Basic characteristics

The basic characteristics of the study subjects are shown in Table 1. After the Bonferroni correction, SBP, TG, and FBS levels were significantly higher in males than females. Whereas, the significantly higher concentrations of LDL-C were observed in females compared to males.

3.2. Allele, and genotype frequencies of CELSR2 (rs629301), APOB100 (rs1367117), ABCG5/8 (rs6544713), LDLR (rs6511720), and APOE (rs429358, rs7412) polymorphisms

The allele and genotype frequencies of *CELSR2* (rs629301), *APOB100* (rs1367117), *ABCG5/8* (rs6544713), *LDLR* (rs6511720), and *APOE* (rs429358, rs7412) polymorphisms are presented in Table 2. The distribution of genotypes of all polymorphisms was in Hardy–Weinberg equilibrium in total subjects, males, and females. After the Bonferroni correction, there was no significantly different in genotype frequencies between males, and females.

3.3. CELSR2 (rs629301), APOB100 (rs1367117), ABCG5/8 (rs6544713), LDLR (rs6511720), and APOE (rs429358, rs7412) polymorphisms, and their association with serum lipids

The basic characteristics according to *CELSR2* (rs629301), *APOB100* (rs1367117), *ABCG5/8* (rs6544713), *LDLR* (rs6511720), and *APOE* (rs429358, rs7412) are shown in Table 3, Table 4, and Table 5. Although the TC, and/or LDL-C were not significantly different among *APOB100* AA, AG, and GG genotypes in total subjects, and females after the Bonferroni correction, TC levels were significantly higher in *APOB100* AA genotype compared with GG (p = 0.007), or AA + AG genotypes (p = 0.007) in total subjects (Table 3). In addition, the significantly higher concentrations of TC and LDL-C were observed in *APOE4* carriers (E3E4, and E4E4 genotypes) compared to *APOE2* carriers (E2E2, E2E3, and E2E4 genotypes) in total subjects (p < 0.001, and p < 0.001, respectively), males (p = 0.004, and p = 0.003, respectively), and females (p = 0.005, and p = 0.002, respectively). The significantly higher concentrations of TC were also observed in *APOE4* carriers (E4E4 genotype) compared to *APOE3* carriers in females (p = 0.005). Whereas, the significantly lower concentrations of TC and LDL-C were observed in *APOE2* carriers (E2E3, E2E2, and E2E4 genotypes) compared to *APOE3* carriers in total subjects (p = 0.006, and p = 0.006, respectively), and males (p = 0.006, and p = 0.003, respectively) (Table 5). Furthermore, TC (p for trend < 0.001), and LDL-C (p for trend < 0.001) levels increased in the order E2E2, E2E4, E2E3, E3E3, E3E4, and E4E4 in total subjects, and females (Table S4). There was no association between *CELSR2* (rs629301), *ABCG5/8* (rs6544713), and *LDLR* (rs6511720) polymorphisms and serum lipids (Table 3, and Table 4).

3.4. Genetic risk scores, and their association with serum lipid levels

Due to the effect of *APOB100*, and *APOE* genotypes on the levels of TC, and LDL-C, genetic risk scores from each risk allele of these

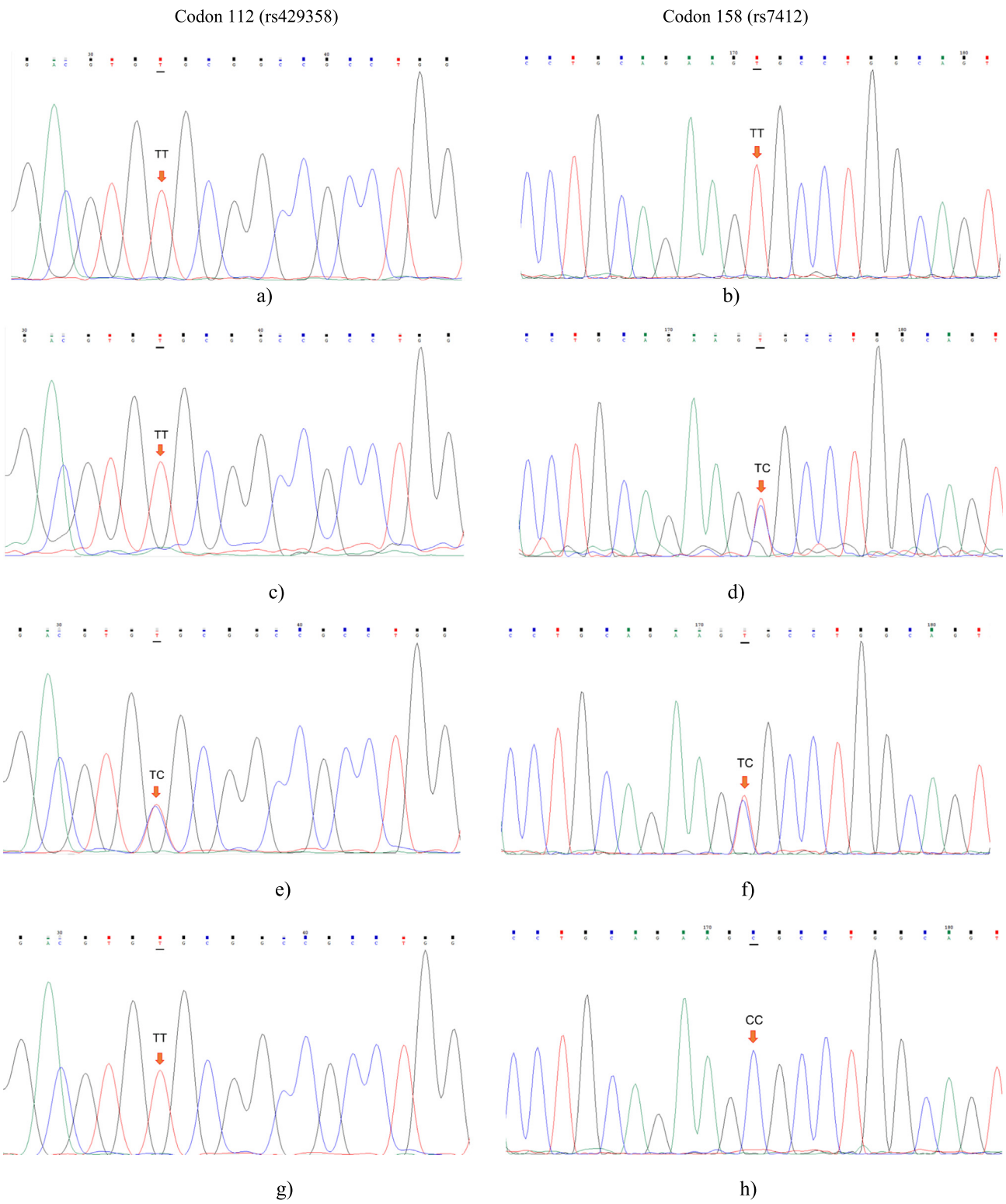


Fig. 2. DNA sequencing for *APOE* genotyping. Sequence chromatogram indicates E2E2 (a-b), E2E3 (c-d), E2E4 (e-f), E3E3 (g-h), E3E4 (i-j), and E4E4 (k-l) genotypes, respectively.

two polymorphisms were calculated. The cumulative effects of *APOB100*, and *APOE* risk alleles on serum lipids are shown in Table 6, and Fig. 3. The concentrations of TC, and LDL-C were significantly increased with the genetic risk scores of *APOB100*, and *APOE* polymorphisms (the score from −2 to 4) in total subjects ($p < 0.001$, and $p < 0.001$, respectively), and females ($p = 0.002$, and $p < 0.001$, respectively). The concentrations of TC, and LDL-C were significantly increased from 153.50 mg/dL, and 66 mg/dL for those without these risk alleles to 285.50 mg/dL, and 202.90 mg/dL for those with these risk alleles in total subjects, respectively. Moreover, the concentrations of TC ($p < 0.001$), and LDL-C ($p < 0.001$) were significantly higher in the combination of

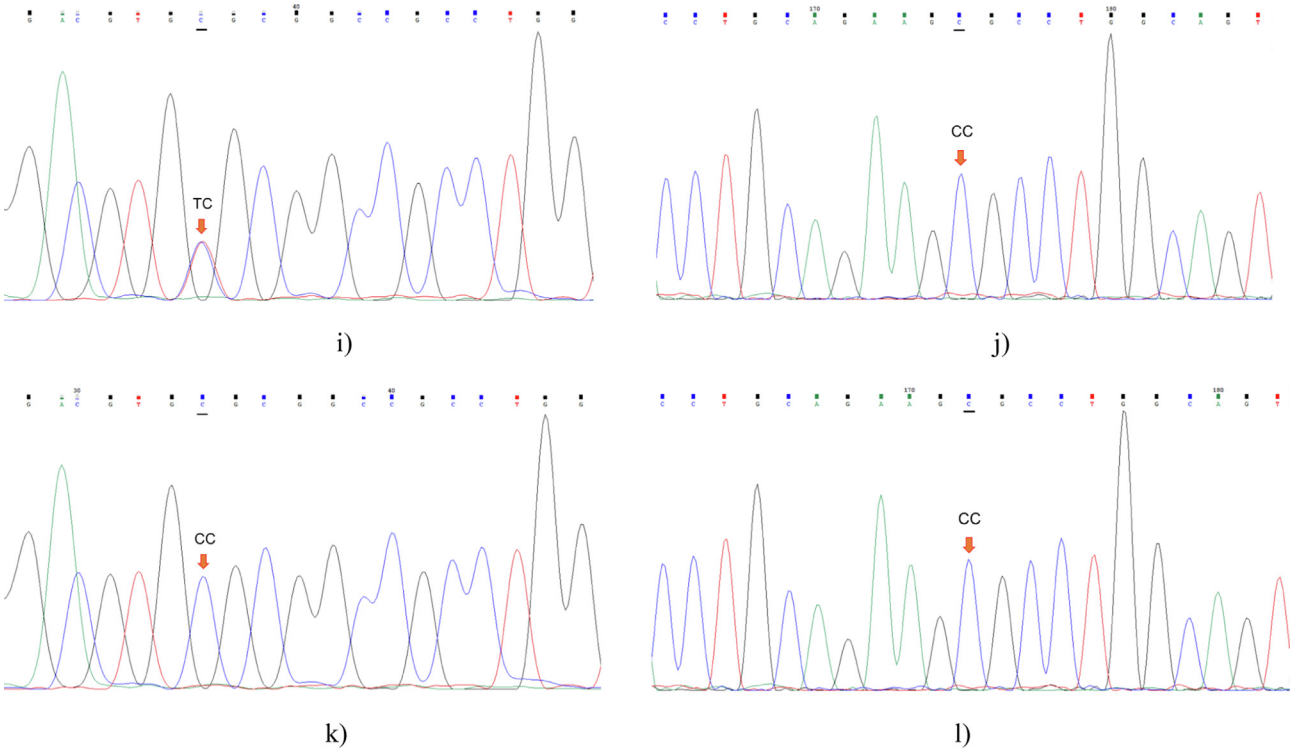


Fig. 2 (*continued*)

Table 1
The basic characteristics of the study subjects.

Variables	Total (n = 459)	Male (n = 184)	Female (n = 275)	p-value*
Age (years)	50.06 ± 11.87	51.65 ± 12.30	48.99 ± 11.47	0.012
BMI (kg/m^2)	23.89 ± 3.73	23.80 ± 3.29	23.95 ± 3.99	0.657
SBP (mmHg)	130.73 ± 19.04	133.58 ± 18.21	128.83 ± 19.38	0.003**
DBP (mmHg)	80.71 ± 11.84	81.09 ± 11.73	80.46 ± 11.92	0.694
TC (mg/dL)	218.51 ± 46.60	212.02 ± 43.29	222.85 ± 48.28	0.020
TG (mg/dL)	116.46 ± 64.15	128.81 ± 66.22	108.19 ± 61.48	<0.001**
HDL-C (mg/dL)	55.34 ± 14.44	54.48 ± 16.10	55.92 ± 13.21	0.079
LDL-C (mg/dL)	141.53 ± 40.53	132.93 ± 40.86	147.29 ± 39.34	0.001**
FBS (mg/dL)	95.06 ± 24.88	103.67 ± 33.92	89.27 ± 13.46	<0.001**

Data are presented as mean ± S.D.
BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; TC, total cholesterol; TG, triglyceride; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; FBS, fasting blood sugar.
* p-value analyzed by Student *t*-test, or Mann-Whitney *U* test.
** Significance at p < 0.008 (0.05/6) after Bonferroni correction.

genetic risk scores (1, 2, 3, and 4) compared with the combination of genetic risk scores (-2, −1, and 0) of *APOB100*, and *APOE* polymorphisms in total subjects (Table S5, Fig. 4). Whereas, there was no relationship between genetic risk scores, and the levels of TG, and HDL-C in total subjects, males, and females. Furthermore, the concentrations of TC (p for trend < 0.001), and LDL-C (p for trend < 0.001) were also increased from the lowest level in non-*APOE4* + *APOB100* AG + GG carriers to the highest level in *APOE4* + *APOB100* AA carriers in total subjects, and females (Table S6-S8, Figure S1).

4. Discussion

In this study, we found that *APOB100*, and *APOE* polymorphisms were associated with serum lipids among Thai subjects. Whereas, *CELSR2, ABCG5/8*, and *LDLR* polymorphisms were not related to serum lipids in this study. In addition, this is the first study that identified the cumulative effect of the risk alleles of *APOB100*, and *APOE* polymorphisms on serum lipids among Thai subjects.

We found that the minor allele frequencies (MAFs) of *CELSR2* (rs629301), *APOB100* (rs1367117), *ABCG5/8* (rs6544713), and *LDLR* (rs6511720) polymorphisms were 8.28 %, 15.58 %, 5.23 %, and 5.23 %, respectively. Similarly, the MAF of *CELSR2* (rs629301) polymorphism was 7.06 %, 6.04 %, and 6.10 % [https://www.ncbi.nlm.nih.gov/snp] in Japanese, Korean, and Vietnamese populations, respectively. Moreover, the MAF of the *APOB100* (rs1367117) population was 14.78 %, and 15.27 % in Asian (ExAC study), and global (HapMap study) populations [https://www.ncbi.nlm.nih.gov/snp], respectively. In contrast, the MAFs of *CELSR2* (rs629301), and *APOB100* (rs1367117) polymorphisms were higher in European (21.56 %, and 30.41 %, respectively), and African American populations (35.74 %, and 10.42 %, respectively) [https://www.ncbi.nlm.nih.gov/snp].

Table 2
Genotype, and allele frequencies of the gene polymorphisms.

Polymorphisms		Total n (%)	Male n (%)	Female n (%)	p-value Male vs Female
CELSR2 (rs629301)					
Genotype	TT	383 (83.44 %)	152 (82.61 %)	231 (84 %)	0.694 (χ^2 = 0.154)
	TG	76 (16.56 %)	32 (17.39 %)	44 (16 %)	
Allele	T	842 (91.72 %)	336 (91.30 %)	506 (92 %)	0.798 (χ^2 = 0.141)
	G	76 (8.28 %)	32 (8.70 %)	44 (8 %)	
HWE (P-value)		0.053	0.196	0.149	
APOB100 (rs1367117)					
Genotype	GG	327 (71.24 %)	139 (75.54 %)	188 (68.36 %)	0.014 (χ^2 = 8.581)
	AG	121 (26.36 %)	45 (24.46 %)	76 (27.64 %)	
	AA	11 (2.40 %)	0 (0 %)	11 (4 %)	
Allele	G	775 (84.42 %)	323 (87.77 %)	452 (82.18 %)	0.018 (χ^2 = 5.553)
	A	143 (15.58 %)	45 (12.23 %)	98 (17.82 %)	
HWE (P-value)		0.961	0.059	0.350	
ABCG5/8 (rs6544713)					
Genotype	CT	48 (10.5 %)	18 (9.78 %)	30 (10.91 %)	0.699 (χ^2 = 0.149)
	CC	411 (89.5 %)	166 (90.22 %)	245 (89.09 %)	
Allele	T	48 (5.23 %)	18 (4.89 %)	30 (5.45 %)	0.623 (χ^2 = 0.242)
	C	870 (94.77 %)	350 (95.11 %)	520 (94.55 %)	
HWE P-value		0.237	0.485	0.339	
LDLR (rs6511720)					
Genotype	CT	48 (10.5 %)	22 (11.96 %)	26 (9.45 %)	0.391 (χ^2 = 0.737)
	CC	411 (89.5 %)	162 (88.04 %)	249 (90.55 %)	
Allele	T	48 (5.23 %)	22 (5.98 %)	26 (4.73 %)	0.480 (χ^2 = 0.499)
	C	870 (94.77 %)	346 (94.02 %)	524 (95.27 %)	
HWE (P-value)		0.237	0.388	0.411	
APOE (rs429358, rs7412)					
Genotype	E2/E2	6 (1.31 %)	1 (0.54 %)	5 (1.82 %)	0.183 (χ^2 = 7.554)
	E2/E3	68 (14.81 %)	26 (14.13 %)	42 (15.27 %)	
	E2/E4	13 (2.83 %)	9 (4.89 %)	4 (1.45 %)	
	E3/E3	253 (55.12 %)	106 (57.61 %)	147 (53.45 %)	
	E3/E4	108 (23.53 %)	38 (20.65 %)	70 (25.45 %)	
	E4/E4	11 (2.40 %)	4 (2.17 %)	7 (2.55 %)	
Allele	E2	93 (10.13 %)	37 (10.05 %)	56 (10.18 %)	0.904 (χ^2 = 202)
	E3	682 (74.29 %)	276 (75 %)	406 (73.82 %)	
	E4	143 (15.58 %)	55 (14.95 %)	88 (16 %)	
HWE (P-value)		0.907	0.391	0.185	

HWE; Hardy-Weinberg equilibrium.
* Significance at p < 0.008 (0.05/6) after Bonferroni correction.

Compared to our results, the MAFs of *ABCG5/8* (rs6544713), and *LDLR* (rs6511720) polymorphisms were lower in the Vietnamese (2.90 %, and 1.90 %, respectively) population [https://www.ncbi.nlm.nih.gov/snp], but MAFs of *ABCG5/8* (rs6544713), and *LDLR* (rs6511720) polymorphisms were higher in European (32.20 %, and 11.76 %, respectively), and African American (16.85 %, and 13.64 %, respectively) populations [https://www.ncbi.nlm.nih.gov/snp]. The frequencies of E2, E3, and E4 alleles were 10.13 %, 74.29 %, and 15.58 %, respectively in this study. Similarly, our results were consistent with the previous studies in Thai population (E2 6.64–7.11 %, E3 76.35–79.56 %, E4 13.33–17.01 %) (Wanmasae et al. 2017, Srirojnopkun et al. 2018). Whereas, the *APOE4* allele from these findings was higher than Saudi (5.5–11.5 %) (Almigbal et al. 2018), Han Chinese (7.5 %) (Han et al. 2016), Taiwanese (7.5 %) (Vasunilashorn et al. 2013), Korean (9 %) (Shin et al. 2014), and Japanese (10.5 %) (Arai et al. 2007) populations, but lower than Malaysian (20.20 %) (Wei et al. 2015), Swedish (20.30 %) (Eggertsen et al. 1993), and Norwegians (19.8 %) (Kumar et al. 2002) populations.

In this study, we found that there was no association between *CELSR2* (rs629301), *ABCG5/8* (rs6544713), and *LDLR* (rs6511720) polymorphisms and serum lipids. In contrast, Noto et al. showed that the *CELSR2* (rs629301) TT genotype was associated with increased levels of LDL-C, non-HDL-C, apoB, apoE, and apoCIII, and decreased levels of HDL-C in coronary artery disease (CAD) patients in Italy (Noto et al. 2021). In addition, *ABCG5/8*

Table 3
Biochemical parameters according to *CELSR2* (rs629301), and *APOB100* (rs1367117) polymorphisms.

	BMI (kg/m^2)	SBP (mmHg)	DBP (mmHg)	TC (mg/dL)	TG (mg/dL)	HDL-C (mg/dL)	LDL-C (mg/dL)	FBS (mg/dL)
CELSR2 (rs629301)								
Total								
TT	23.85 ± 3.62	131.14 ± 19.34	81.20 ± 11.98	218.42 ± 47.54	115.92 ± 62.55	55.13 ± 14.23	142.10 ± 40.57	94.60 ± 22.80
TG	24.04 ± 4.22	128.71 ± 17.48	78.24 ± 10.84	218.95 ± 41.81	119.18 ± 72.08	56.46 ± 15.51	138.69 ± 40.48	97.34 ± 33.52
p-value [a]	0.953	0.327	0.043	0.768	0.718	0.468	0.404	0.701
Male								
TT	23.76 ± 3.35	133.60 ± 17.80	78.88 ± 11.70	211.22 ± 43.84	128.05 ± 66.44	53.75 ± 15.60	131.32 ± 42.07	103.53 ± 30.96
TG	23.96 ± 3.02	133.47 ± 20.32	81.55 ± 11.72	215.81 ± 41.01	132.41 ± 66.05	57.97 ± 18.15	133.27 ± 40.74	104.31 ± 46.09
p-value [a]	0.762	0.818	0.240	0.587	0.773	0.167	0.808	0.291
Female								
TT	23.92 ± 3.80	125.25 ± 14.35	80.97 ± 12.16	223.16 ± 49.35	107.93 ± 58.64	56.03 ± 13.20	147.05 ± 39.48	88.70 ± 12.05
TG	24.09 ± 4.89	129.52 ± 20.16	77.77 ± 10.28	221.23 ± 42.71	109.57 ± 75.45	55.36 ± 13.39	144.05 ± 38.89	92.27 ± 19.12
p-value [a]	0.723	0.315	0.100	0.513	0.378	0.798	0.444	0.271
APOB100 (rs1367117)								
Total								
Codominant model								
AA	24.83 ± 3.31	142.09 ± 18.23	87.09 ± 19.10	258.82 ± 49.72[b,c*]	111.55 ± 45.07	60.27 ± 13.15	176.18 ± 45.60[b, c]	98.27 ± 27.09
AG	23.49 ± 3.89	128.44 ± 17.69	79.87 ± 10.89	217.25 ± 48.07	119.88 ± 66.20	54.39 ± 13.71	141.30 ± 40.15	92.63 ± 28.37
GG	24.00 ± 3.68	131.19 ± 19.42	80.81 ± 11.84	217.62 ± 45.48	115.36 ± 64.04	55.53 ± 14.74	140.46 ± 40.11	95.85 ± 23.40
p-value [a]	0.166	0.055	0.398	0.025	0.856	0.368	0.034	0.040
Recessive model								
AA + AG	23.56 ± 3.87	129.59 ± 18.07	80.47 ± 11.86	220.71 ± 49.38	144.20 ± 41.58	119.19 ± 64.61	54.88 ± 13.71	93.10 ± 28.21
GG	24.00 ± 3.68	131.19 ± 19.42	80.81 ± 11.84	217.62 ± 45.48	140.46 ± 40.11	115.35 ± 64.04	55.53 ± 14.74	95.85 ± 23.40
p-value [a]	0.108	0.499	0.525	0.326	0.578	0.939	0.319	0.019
Dominant model								
AA	24.83 ± 3.31	142.09 ± 18.23	87.09 ± 19.10	258.82 ± 49.71	111.55 ± 45.07	60.27 ± 13.15	176.18 ± 45.60	98.27 ± 27.09
AG + GG	23.87 ± 3.74	130.45 ± 18.99	80.55 ± 11.59	217.52 ± 46.14	116.58 ± 64.58	55.22 ± 14.46	140.68 ± 40.08	94.98 ± 24.85
p-value [a]	0.505	0.038	0.315	0.007*	0.876	0.177	0.010	0.698
Over dominant model								
AA + GG	24.02 ± 3.67	131.55 ± 19.46	81.01 ± 12.16	218.96 ± 46.13	115.23 ± 63.46	55.69 ± 14.70	141.62 ± 40.73	95.93 ± 23.48
AG	23.49 ± 3.89	128.44 ± 1769	79.87 ± 10.89	217.25 ± 48.07	119.88 ± 66.20	54.39 ± 13.71	141.30 ± 40.15	92.63 ± 18.37
p-value [a]	0.069	0.156	0.316	0.943	0.604	0.584	0.898	0.011
Male								
AA	–	–	–	–	–	–	–	–
AG	23.39 ± 3.52	129.25 ± 18.26	79.62 ± 11.50	210.89 ± 47.61	130.42 ± 71.55	56.16 ± 17.66	131.38 ± 43.01	100.67 ± 40.52
GG	23.93 ± 3.22	134.95 ± 18.04	81.56 ± 11.81	212.38 ± 41.98	128.29 ± 64.66	53.94 ± 15.59	133.43 ± 40.29	104.64 ± 31.60
p-value [a]	0.337	0.036	0.144	0.841	0.979	0.470	0.771	0.128
Female								
Codominant model								
AA	24.83 ± 3.31	142.09 ± 18.23	87.09 ± 19.10	258.82 ± 49.71[b, c]	111.55 ± 45.07	60.27 ± 13.15	176.18 ± 45.60[b, c]	98.27 ± 27.09
AG	23.57 ± 4.12	127.96 ± 17.46	80.01 ± 10.60	221.01 ± 48.25	113.64 ± 62.47	53.34 ± 10.71	147.17 ± 37.42	87.87 ± 16.21
GG	24.06 ± 3.98	128.40 ± 19.98	80.25 ± 11.87	221.49 ± 47.65	105.79 ± 62.02	56.71 ± 13.99	145.65 ± 39.29	89.32 ± 10.72
p-value [a]	0.331	0.051	0.572	0.050	0.467	0.240	0.058	0.098
Recessive model								
AA + AG	23.67 ± 4.05	129.77 ± 18.08	80.91 ± 12.08	225.79 ± 49.77	113.38 ± 60.33	54.22 ± 11.21	150.84 ± 39.46	89.18 ± 18.07
GG	24.06 ± 3.98	128.40 ± 19.98	80.25 ± 11.87	221.49 ± 47.65	105.79 ± 62.02	56.71 ± 13.99	145.65 ± 39.29	89.32 ± 10.72
p-value [a]	0.279	0.311	0.693	0.369	0.223	0.243	0.359	0.184
Dominant model								
AA	24.83 ± 3.31	142.09 ± 18.23	87.09 ± 19.10	258.82 ± 49.71	111.55 ± 45.07	60.27 ± 13.15	176.18 ± 45.60	98.27 ± 27.09
AG + GG	23.92 ± 4.01	128.27 ± 19.26	80.18 ± 11.50	221.35 ± 47.73	108.05 ± 62.13	55.74 ± 13.21	146.09 ± 38.69	88.90 ± 12.54
p-value [a]	0.465	0.016	0.292	0.015	0.463	0.280	0.020	0.222
Over dominant model								
AA + GG	24.08 ± 3.96	129.16 ± 20.09	80.63 ± 12.41	223.55 ± 48.40	106.11 ± 61.14	56.90 ± 13.95	147.34 ± 40.15	89.81 ± 12.25
AG	23.57 ± 4.12	127.96 ± 17.46	80.01 ± 10.60	221.01 ± 48.25	113.64 ± 62.47	53.34 ± 10.71	147.17 ± 37.42	87.87 ± 16.21
p-value [a]	0.174	0.991	0.959	0.892	0.372	0.154	0.803	0.055

Data are given mean ± S.D.
* Significance at p < 0.008 (0.05/6) after Bonferroni correction.
[a] p-value analyzed by Kruskal-Wallis test, or Mann-Whitney *U* test.
[b] AA vs AG, *p*-value < 0.05, Student *t*-test, or Mann-Whitney *U* test.
[c] AA vs GG, *p*-value < 0.05, Student *t*-test, or Mann-Whitney *U* test.

(rs6544713) polymorphism was found related to TC, and LDL-C concentrations in the previous GWAS studies in European populations (Aulchenko et al. 2008, Kathiresan et al. 2009b, Teslovich et al. 2010). Moreover, minor allele or T allele of *LDLR* (rs6511720) polymorphism was related to decreased levels of LDL-C in African ancestry (Miljkovic et al. 2010), and European population (Teslovich et al. 2010). *LDLR* (rs6511720) polymorphism was also related to a lower CAD (Teslovich et al. 2010, Aulchenko et al. 2008, Kathiresan et al. 2008), and myocardial infarction (MI) risks (Anand et al. 2009). Nevertheless, *APOB100* (rs1367117) polymorphism was related to increased concentrations of TC, and LDL-C in this study. Similarly, *APOB100* (rs1367117) polymorphism was associated with apoB levels in the Chinese population (Andreotti et al. 2009), as well as, TC, LDL-C, non-HDL-C, and apoB levels in the GWAS (Chasman et al. 2009, Teslovich et al. 2010). *APOB100* (rs1367117) was a nonsynonymous polymorphism. It resulted in the substitution of amino acid from threonine to isoleucine at position 71 (T71I). This polymorphism is located in the domains that directly interacted with microsomal triglyceride transfer protein (MTTP), and the protein disulfide isomerase for lipidation of the nascent apolipoprotein B (Bradbury et al. 1999). Although, the mechanism of the effect of

Table 4
Biochemical parameters according to *ABCG5/8* (rs6544713), and *LDLR* (rs6511720) polymorphisms.

	BMI (kg/m^2)	SBP (mmHg)	DBP (mmHg)	TC (mg/dL)	TG (mg/dL)	HDL-C (mg/dL)	LDL-C (mg/dL)	FBS (mg/dL)
ABCG5/8 (rs6544713)								
Total								
CT	24.82 ± 3.76	132.73 ± 24.47	81.27 ± 13.64	216.75 ± 52.57	103.98 ± 56.36	53.54 ± 13.38	148.74 ± 36.91	97.46 ± 33.70
CC	23.78 ± 3.72	130.50 ± 18.32	80.65 ± 11.62	218.71 ± 45.92	117.91 ± 64.91	55.55 ± 14.56	140.69 ± 40.89	94.78 ± 23.67
p-value [a]	0.073	0.831	0.981	0.819	0.142	0.304	0.172	0.961
Male								
CT	24.82 ± 2.55	133.44 ± 17.68	80.17 ± 13.09	198.33 ± 55.96	107.17 ± 57.65	56.00 ± 15.78	131.53 ± 33.49	110.89 ± 51.30
CC	23.70 ± 3.35	133.59 ± 18.32	81.19 ± 11.62	213.50 ± 41.63	131.16 ± 66.81	54.32 ± 16.17	133.08 ± 41.67	102.89 ± 31.61
p-value [a]	0.208	0.946	0.823	0.159	0.199	0.616	0.879	0.760
Female								
CT	24.81 ± 4.34	132.30 ± 28.04	81.93 ± 14.13	227.80 ± 48.02	102.07 ± 56.48	52.07 ± 11.76	159.07 ± 35.44	89.40 ± 10.90
CC	23.84 ± 3.95	128.40 ± 18.06	80.28 ± 11.64	222.24 ± 48.38	108.94 ± 62.14	56.39 ± 13.32	145.85 ± 39.62	89.26 ± 13.76
p-value [a]	0.247	0.840	0.870	0.345	0.618	0.063	0.057	0.702
LDLR (rs6511720)								
Total								
CT	23.68 ± 3.32	132.29 ± 23.48	80.81 ± 12.77	213.08 ± 48.35	125.67 ± 69.86	52.75 ± 12.19	138.04 ± 38.37	97.04 ± 33.92
CC	23.91 ± 3.78	130.55 ± 18.48	80.70 ± 11.74	219.14 ± 46.41	115.38 ± 63.46	55.65 ± 14.66	141.94 ± 40.80	94.82 ± 23.64
p-value [a]	0.975	0.973	0.894	0.523	0.260	0.225	0.540	0.694
Male								
CT	23.62 ± 3.01	131.45 ± 14.55	79.68 ± 11.24	206.55 ± 43.66	137.95 ± 89.34	53.64 ± 14.76	126.88 ± 36.73	106.27 ± 47.84
CC	23.82 ± 3.34	133.87 ± 18.67	81.28 ± 11.82	212.76 ± 43.32	127.57 ± 62.69	54.60 ± 16.31	133.75 ± 41.43	103.31 ± 31.76
p-value [a]	0.796	0.674	0.508	0.529	0.985	0.824	0.461	0.262
Female								
CT	23.74 ± 3.62	133.00 ± 29.29	81.80 ± 14.12	218.62 ± 52.19	115.27 ± 47.17	52.00 ± 9.75	147.48 ± 37.85	89.23 ± 10.09
CC	23.97 ± 4.04	128.39 ± 18.06	80.32 ± 11.70	223.29 ± 47.95	107.45 ± 62.82	56.33 ± 13.47	147.27 ± 39.57	89.28 ± 13.78
p-value [a]	0.995	0.006*	0.714	0.832	0.118	0.160	0.928	0.790

Data are given mean ± S.D.
* Significance at p < 0.008 (0.05/6) after Bonferroni correction.
[a] p-value analyzed by Student *t*-test, or Mann-Whitney *U* test.

Table 5
Biochemical parameters according to *APOE* allele.

	BMI (kg/m^2)	SBP (mmHg)	DBP (mmHg)	TC (mg/dL)	TG (mg/dL)	HDL-C (mg/dL)	LDL-C (mg/dL)	FBS (mg/dL)
APOE								
Total								
E2	23.85 ± 3.84	128.51 ± 18.55	78.15 ± 10.95	202.91 ± 51.95[b*,c*]	115.46 ± 63.27	55.58 ± 14.89	125.80 ± 45.92[b*,c*]	94.46 ± 20.76
E3	23.87 ± 3.78	130.46 ± 19.61	80.51 ± 11.65	216.78 ± 41.86 [d*]	114.74 ± 65.22	55.39 ± 14.38	140.60 ± 35.11 [d*]	95.11 ± 27.26
E4	23.93 ± 3.59	132.49 ± 18.19	82.53 ± 12.44	230.57 ± 49.28	120.30 ± 62.88	120.30 ± 62.88	152.14 ± 44.08	95.30 ± 22.25
p-value [a]	0.967	0.272	0.159	<0.001*	0.404	0.947	<0.001*	0.184
Male								
E2	23.31 ± 2.52	130.33 ± 17.54	79.96 ± 9.10	189.26 ± 40.35[b*,c*]	126.15 ± 65.46	53.44 ± 17.68	110.60 ± 35.58[b*,c*]	103.93 ± 29.90
E3	23.72 ± 3.51	135.09 ± 19.42	81.46 ± 12.45	212.82 ± 39.17	132.44 ± 68.37	54.42 ± 15.51	133.90 ± 36.28	104.53 ± 36.31
E4	24.20 ± 3.20	132.20 ± 15.83	80.90 ± 11.59	222.39 ± 48.99	122.67 ± 62.73	55.16 ± 16.74	142.74 ± 48.16	101.75 ± 31.21
p-value [a]	0.521	0.493	0.903	0.005*	0.674	0.863	0.004*	0.973
Female								
E2	24.16 ± 4.42	127.43 ± 19.22	77.11 ± 11.85[c]	210.74 ± 56.49[c*]	109.32 ± 61.85	56.81 ± 13.08	134.54 ± 49.16[b,c*]	89.02 ± 9.77
E3	23.98 ± 3.97	127.13 ± 19.13	79.81 ± 11.02	219.63 ± 43.60 [d*]	101.98 ± 59.92	56.08 ± 12.52	145.43 ± 33.53 [d]	88.27 ± 14.83
E4	23.75 ± 3.82	132.68 ± 19.61	83.56 ± 12.91	235.72 ± 49.06	118.81 ± 63.33	55.11 ± 12.82	158.06 ± 40.50	91.23 ± 12.59
p-value [a]	0.820	0.095	0.057	0.003*	0.042	0.776	0.001*	0.026

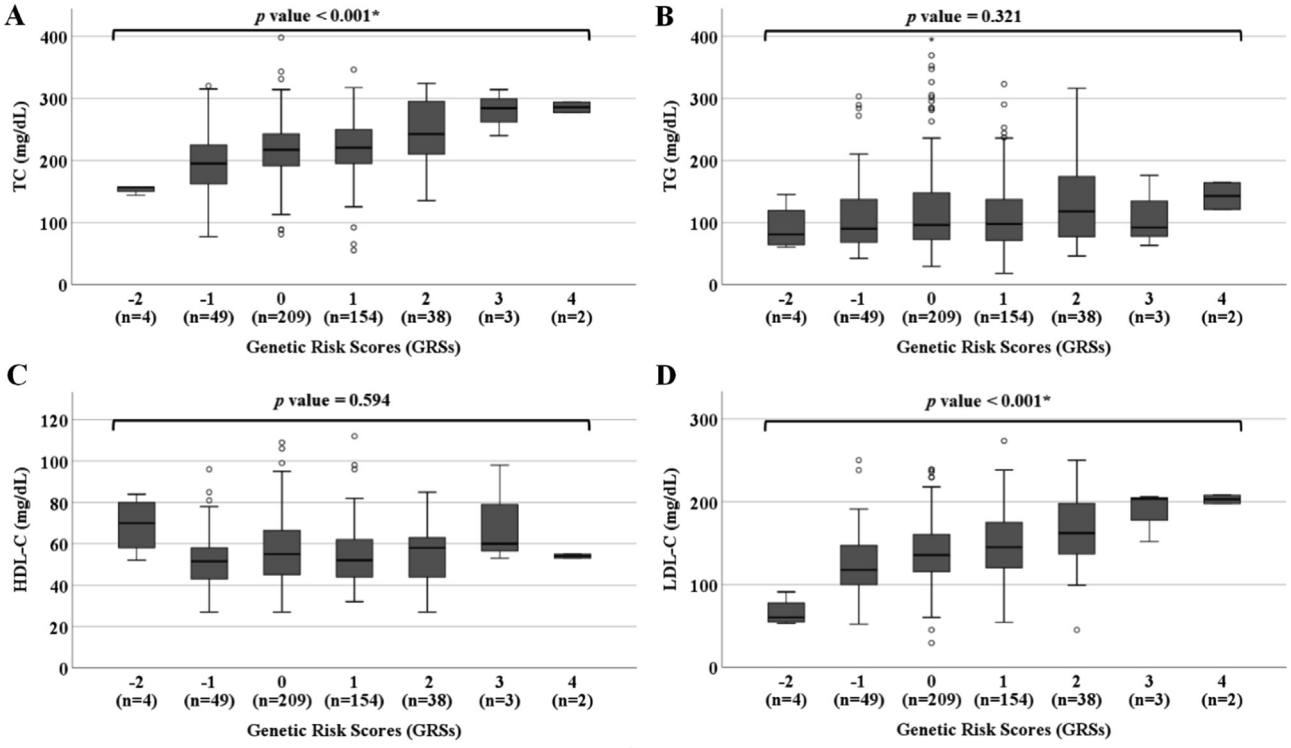
Data are given mean ± S.D.
* Significance at p < 0.008 (0.05/6) after Bonferroni correction.
[a] p-value analyzed by one-way ANOVA, or Kruskal-Wallis test.
[b] E2 vs E3, *p*-value < 0.05, Student *t*-test, or Mann-Whitney *U* test.
[c] E2 vs E4, *p*-value < 0.05, Student *t*-test, or Mann-Whitney *U* test.
[d] E3 vs E4, *p*-value < 0.05, Student *t*-test, or Mann-Whitney *U* test.

APOB100 T71I on the increased levels of TC, and LDL-C is still unclear, it is hypothesized that this polymorphism may promote the synthesis of LDL, and decrease the LDL uptake into the hepatocyte. Furthermore, *APOE* polymorphism was related to TC, and LDL-C concentrations in this study. Previous studies also supported that *APOE4* allele was related to the increased TC, and LDL-C concentrations in Thai hypercholesterolemia, and type 2 diabetes patients (Wanmasae et al. 2017, Srirojnopkun et al. 2018). Our results were agreed with the studies in urban Brazilian individuals (Alvim et al. 2010), Mexican adolescents (Medina-Urrutia et al. 2004), and healthy Chinese individuals (Liang et al. 2009) in which *APOE4* allele was related to increased TC, and LDL-C concentrations. In addition, the *meta*-analysis also supported that *APOE4* allele increased the risk for hyperlipidemia in both Asians, and Caucasians populations (Zhao et al. 2021). In contrast, the *APOE4* allele showed significantly lower concentrations of TC compared with *APOE3* allele in women from Northern Chile (Gálvez et al. 2021). Moreover, *APOE* polymorphism was not found associated with serum lipids in Saudi populations (Almigbal et al. 2018). Altogether, we suggested that the different allele frequencies of these polymorphisms among various studies, and the inconsistent results of the association between these polymorphisms, and serum lipids may be due to the different populations, and ethnicities, as well as, the number of the study subjects. Finally, we also demonstrated the cumulative effects of the *APOB100*, and *APOE* risk alleles on serum TC, and LDL-C concentrations in the present study.

Table 6
Cumulative effects of the risk alleles of *APOB100*, and *APOE* polymorphisms on serum lipids.

Genetic risk scores	−2	−1	0	1	2	3	4	B (95 % CI)	p-value*
Genotyping	*APOE* E2E2 + *APOB100* GG	*APOE* E2E2 + *APOB100* AG, or *APOE* E2E3 + *APOB100* GG	*APOE* E2E4 + *APOB100* GG, or *APOE* E3E3 + *APOB100* GG	*APOE* E3E3 + *APOB100* AG, or *APOE* E2E4 + *APOB100* AG, or *APOE* E3E4 + *APOB100* GG	*APOE* E4E4 + *APOB100* GG, or *APOE* E3E4 + *APOB100* AG	*APOE* E3E4 + *APOB100* AA	*APOE* E4E4 + *APOB100* AA		
TC (mg/dL)									
Total	153.50 ± 6.35	199.00 ± 52.85	217.33 ± 42.47	219.19 ± 46.09	244.45 ± 46.51	279.33 ± 37.22	285.50 ± 12.02	12.886 (8.115–17.656)	<0.001**
Male	156	189.56 ± 42.25	211.08 ± 38.28	215.05 ± 46.28	240.92 ± 51.03	–	–	12.690 (4.762-20.618)	0.012
Female	152.67 ± 7.51	205.07 ± 58.59	222.40 ± 45.11	221.62 ± 46.04	246.28 ± 44.98	279.33 ± 37.22	285.50 ± 12.02	12.520 (6.568–18.471)	0.002**
TG (mg/dL)									
Total	91.75 ± 38.35	114.24 ± 67.59	118.20 ± 68.08	111.34 ± 57.42	131.87 ± 67.19	110.33 ± 58.69	142.50 ± 30.41	3.381 (-3.311–10.073)	0.321
Male	94.00	129.33 ± 71.48	130.74 ± 66.19	121.49 ± 62.50	148.77 ± 79.13	–	–	1.256 (-11.319–13.831)	0.844
Female	91.00 ± 46.94	104.54 ± 64.39	108.02 ± 68.17	105.38 ± 53.65	123.08 ± 59.95	110.33 ± 58.69	142.50 ± 30.41	3.838 (-3.899–11.575)	0.330
HDL-C (mg/dL)									
Total	69.00 ± 14.00	52.61 ± 13.74	56.71 ± 15.56	53.70 ± 12.96	55.16 ± 12.62	70.33 ± 24.21	54.00 ± 1.41	−0.415 (-1.945–1.114)	0.594
Male	84.00	48.28 ± 11.35	55.59 ± 17.44	54.26 ± 14.71	53.69 ± 15.46	–	–	0.257 (-2.798–3.313)	0.868
Female	64.00 ± 12.00	55.39 ± 14.59	57.62 ± 13.86	53.37 ± 11.88	55.92 ± 11.15	70.33 ± 24.21	54.00 ± 1.41	−0.733 (-2.431–0.965)	0.396
LDL-C (mg/dL)									
Total	66.00 ± 17.30	126.10 ± 44.63	138.65 ± 37.36	145.13 ± 38.24	162.87 ± 44.51	187.07 ± 30.57	202.90 ± 7.21	12.171 (8.079-16.264)	<0.001**
Male	53	115.45 ± 35.45	130.28 ± 38.23	138.68 ± 39.67	157.45 ± 55.12	–	–	12.644 (5.207–20.082)	0.009
Female	70.33 ± 18.34	132.95 ± 49.04	145.45 ± 35.35	148.92 ± 37.05	165.68 ± 38.87	187.07 ± 30.57	202.90 ± 7.21	11.572 (6.742–16.402)	<0.001**

*Linear regression analysis adjusted by age, and gender.
** Significance at $p < 0.008$ (0.05/6) after Bonferroni correction.



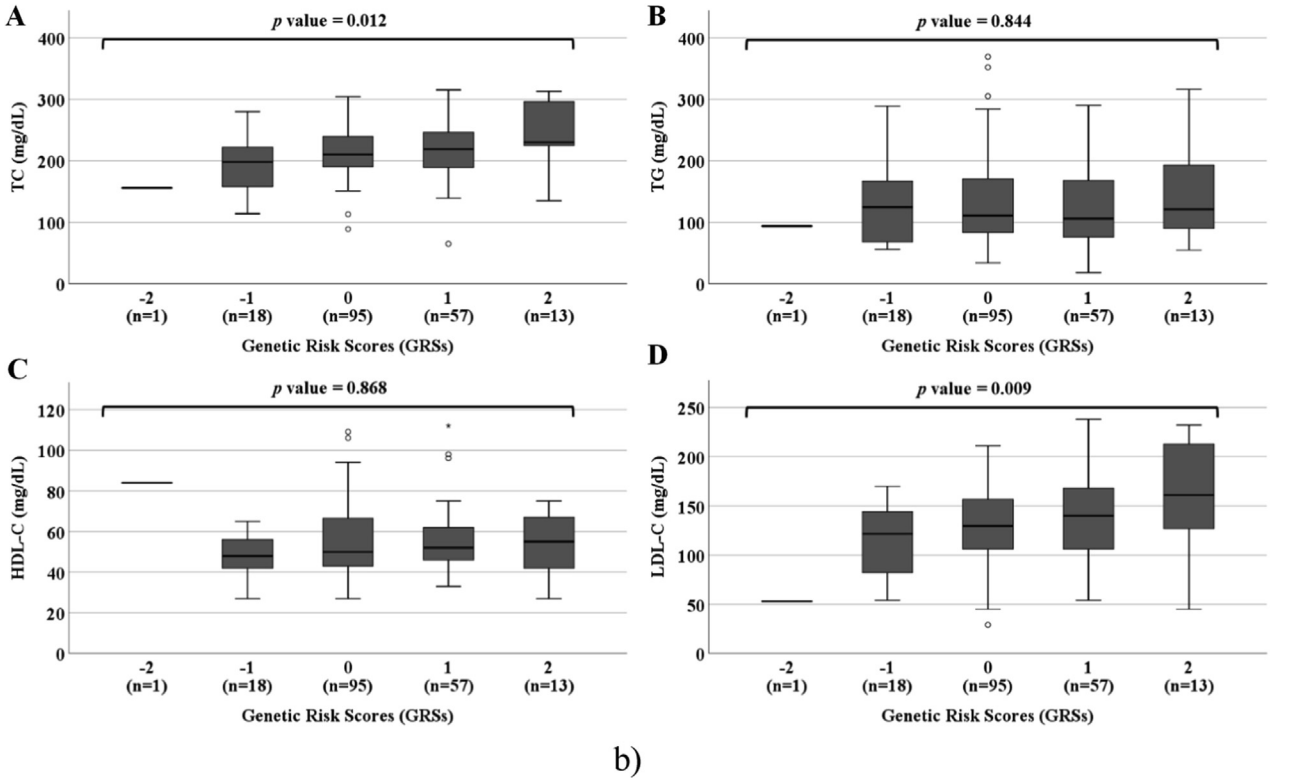
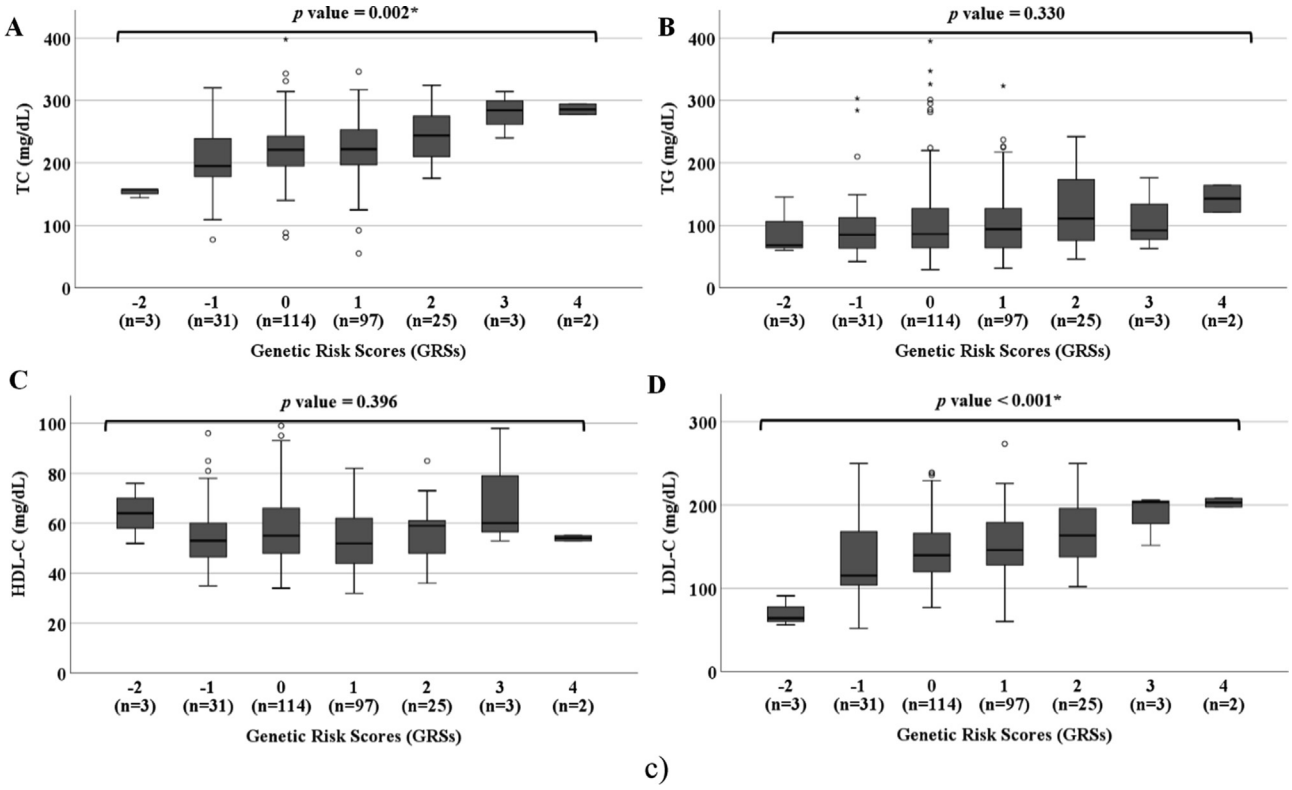


Fig. 3. Box plot showing serum lipids, and genetic risk scores of *APOB100*, and *APOE* polymorphisms in total subjects (a), males (b), and females (c). Horizontal line in the box is the median, with boxes extending from 25 % to 75 % of values (interquartile range). The outliers are shown as dots. * Significance at p < 0.008 (0.05/6) after Bonferroni correction.

TC, and LDL-C concentrations were significantly increased from those without risk alleles to those with risk alleles of *APOB100*, and *APOE* polymorphisms in total subjects, males, and females.

There was a limitation in this study. The AA genotype of *APOB100* (rs1367117) polymorphism was not detected in males. This may be due to the small number of males, or the



c)

Fig. 3 (*continued*)

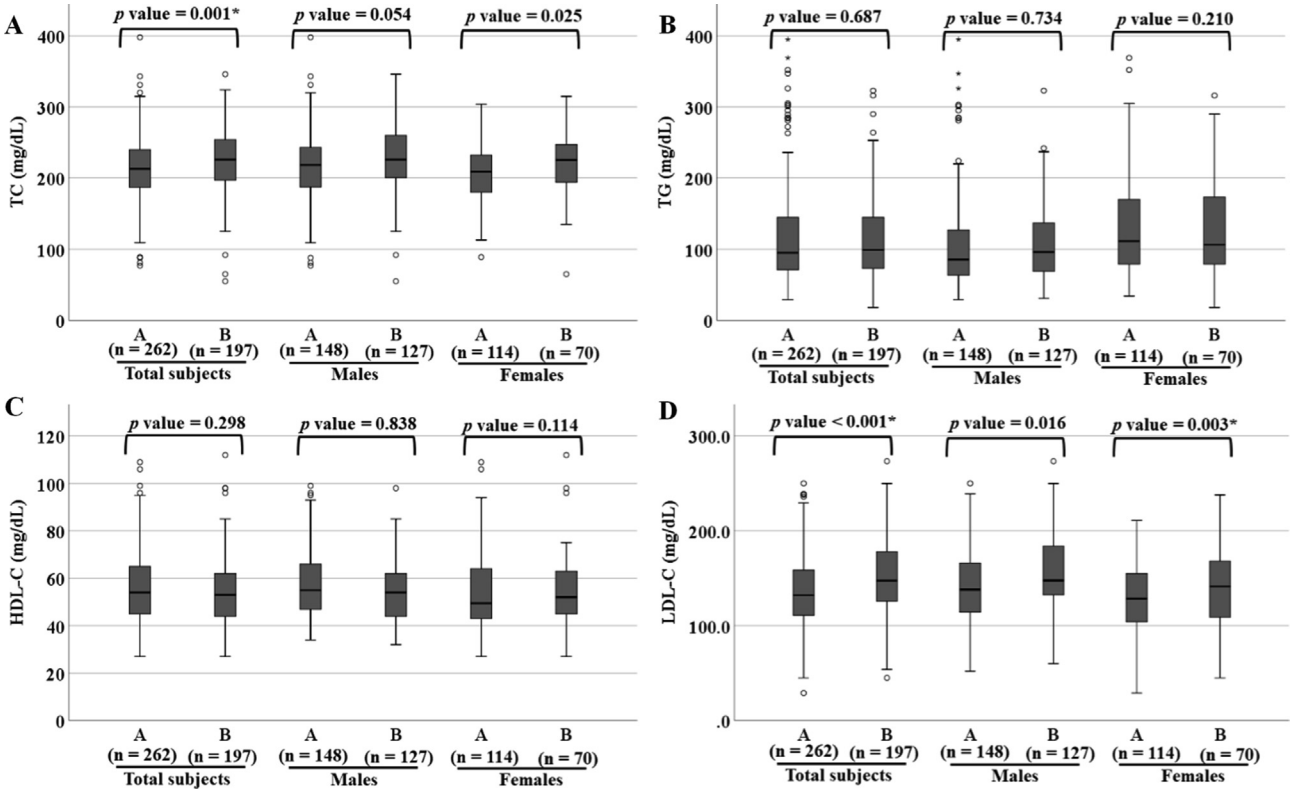


Fig. 4. Box plot showing serum lipids, between the combination of genetic risk scores −2, −1, and 0 (A) compared with the combination of genetic risk scores 1, 2, 3, and 4 of *APOB100*, and *APOE* polymorphisms (B). Horizontal line in the box is the median, with boxes extending from 25 % to 75 % of values (interquartile range). The outliers are shown as dots. * Significance at p < 0.008 (0.05/6) after Bonferroni correction.

gender-specific of *APOB100* (rs1367117) polymorphism among this population. Further study in the larger sample size should be performed to confirm our findings.

In conclusion, *APOB100* (rs1367117), and *APOE* (rs429358, rs7412) but not *CELSR2* (rs629301), *ABCG5/8* (rs6544713), and *LDLR* (rs6511720) polymorphisms were associated with serum lipids. The cumulative risk alleles of *APOB100* (rs1367117), and *APOE* (rs429358, rs7412) polymorphisms could enhance the elevated concentrations of TC, and LDL-C, and they may be used to predict severity of hypercholesterolemia among Thai subjects.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.sjbs.2022.103554.

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