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Genetic association with low concentrations of high density lipoprotein-cholesterol in a pediatric population of the Middle East and North Africa: The CASPIAN-III study

Roya Kelishadi [a], Shaghayegh Haghjooy Javanmard [b, *], Mohammad Hasan Tajadini [b], Marjan Mansourian [a, b, c], Mohammad Esmaeil Motlagh [d], Gelayol Ardalan [a], Matthew Ban [e]

[a] Pediatrics Department, Child Growth and Development Research Center, Isfahan University of Medical Sciences, Isfahan, Iran
[b] Physiology Department, Applied Physiology Research Center, Isfahan University of Medical Sciences, Isfahan, Iran
[c] Biostatistics Department, Faculty of Health, Isfahan University of Medical Sciences, Isfahan, Iran
[d] Pediatrics Department, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran
[e] Blackburn Cardiovascular Genetics Laboratory, Robarts Research Institute, University of Western Ontario, Ontario, Canada

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ABSTRACT

Objective: Depressed high-density lipoprotein cholesterol (HDL-C) is prevalent the Middle East and North Africa. Some studies have documented associations between HDL-C and several single nucleotide polymorphisms (SNPs) in candidate gene polymorphisms.
Methods: We investigated the associations between SNP genotypes and HDL-C levels in Iranian students, aged 10–18 years. Genotyping was performed in 750 randomly selected participants among those with low HDL-C levels (below 5th percentile), intermediate HDL-C levels (5–95th) and high HDL-C levels (above the 95th percentile). Minor allele frequencies (MAFs) of the SNPs of interest were compared between the three HDL-C groups.
Results: The vast majority of pairwise comparisons of MAFs between HDL-C groups were significant. Pairwise comparisons between low and high HDL-C groups showed significant between-group differences in MAFs for all SNPs, except for *APOC3* rs5128. Pairwise comparisons between low and intermediate HDL-C groups showed significant between-group differences in MAFs for all SNPs, except for *APOC3* rs5128 and *APOA1* rs2893157. Pairwise comparisons between intermediate and high HDL-C groups showed significant between-group differences in MAFs for all SNPs, except for *ABCA1 APOC3* rs5128 and *APOA1* rs2893157. After adjustment for confounding factors, including age, sex, body mass index, low physical activity, consumption of saturated fats, and socioeconomic status, *ABCA1* r1587K and CETP A373P significantly increased the risk of depressed HDL-C, and CETP Taq1 had a protective role.
Conclusion: This study replicated several associations between HDL-C levels and candidate gene SNPs from genome-wide associations with HDL-C in Iranians from the pediatric age group.

1. Introduction

Epidemiologic and observational studies have consistently shown that depressed plasma levels of high-density lipoprotein cholesterol (HDL-C) represent an independent inverse predictor of atherosclerotic cardiovascular coronary heart disease (ACVD) risk. Patients with low HDL-C have been suggested to have ACVD risk that is comparable to those with high levels of low-density lipoprotein cholesterol (LDL-C) [1,2]. The mechanism by which HDL-C confers protection against atherosclerosis remains speculative. HDL-C may be involved in reverse cholesterol transport from peripheral tissues, including macrophages in the arterial wall, to the liver [3]. Other potential cardioprotective mechanisms include inhibition of LDL-C oxidation, possibly mediated by paraoxonase, and stabilization of the production of prostacyclin, an important vasodilator and inhibitor of platelet aggregation [4].

* Corresponding author. Applied Physiology Research Center, Isfahan University of Medical Sciences, Hezar-Jarib Avenue, 81676-36954, Isfahan, Iran.
E-mail addresses: shaghayeghhaghjoo@yahoo.com, sh_haghjoo@med.mui.ac.ir (S. Haghjooy Javanmard).

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Several epidemiologic studies have shown a high prevalence of depressed HDL-C in the population of the Middle East and North Africa (MENA) [5–7], even among immigrants from this region to Western countries [8,9]. National studies in Iran revealed a markedly high prevalence of this disturbance in adults [7] as well as in children [10,11]. Moreover, the 5th percentile of serum HDL-C level of Iranian children and adolescents is lower than their European [12] and American counterparts [13], suggesting that the leftward shift in the distribution of this trait among Iranians begins in childhood. Although lifestyle factors may explain, at least in part [11], the high prevalence of this type of dyslipidemia among adults, the very high prevalence among children and adolescents even among normal-weight subjects [14] and in immigrants [15] cannot be explained only by unhealthy lifestyle behaviors.

Genetic association studies have consistently demonstrated that variants in several candidate genes are significant determinants of lipid and HDL-C levels in adults. Many of the earlier candidate genes that were significantly associated with HDL-C on an individual basis have been confirmed in very large genome-wide association studies (GWAS) of adults [16]. However, to the best of our knowledge, no previous nationwide study has investigated associations of candidate gene polymorphisms with low HDL-C in children and adolescents. Therefore, in the present investigation we examine the association of common well-established variants in some GWAS-validated candidate genes with HDL-C levels in a nationally representative sample of Iranian children, as a pediatric population of the MENA region.

2. Methods

2.1. Study population

This project was conducted as a sub-study of the "national survey of school student high risk behaviors" (2009–2010), which was the third survey of the school-based surveillance system entitled Childhood and Adolescence Surveillance and Prevention of Adult Non communicable disease (CASPIAN-III) Study[1]. This school-based nationwide health survey was conducted in 27 provinces in Iran. Ethics committees and other relevant national regulatory organizations approved the study. Our team obtained written informed consent from parents and oral assent from children and adolescents. Details of data collection and sampling are published previously [17], and here we present these in brief.

The main survey included 5528 students aged 10–18 years who were recruited by multistage random cluster sampling from urban and rural areas of 27 provincial counties in Iran. For the current study, we randomly selected 750 frozen whole blood samples from three groups of children and adolescents according to the percentiles of HDL-C; i.e. high HDL-C (above the 95th percentile), intermediate HDL-C (5–95th percentile) and low HDL-C (below 5th percentile). Each group comprised 250 individuals. This sample size was calculated. The physical activity level and socioeconomic status, considered as component variables, were calculated according to the principle component analysis method as previously published from the CASPIAN-III study [11].

2.2. Physical examination and biochemical measurements

A team of trained health care professionals and physicians recorded information, and measured weight, height, waist circumference, and blood pressure under standard protocols using calibrated instruments. Body mass index (BMI) was calculated as the weight (kg) divided by the height squared (m^2). For blood sampling, students were invited to the nearest health center to the school where fasting venous blood was taken. Details of biochemical measurements are reported previously; in summary plasma levels of glucose; lipid profile variables including total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) were measured by autoanalyzer and using Pars Azmoon reagents kit (Tehran, Iran). In each county, the biochemical analysis was performed in the central provincial laboratory that met the standards of the National Reference laboratory, a collaborating center of the World Health Organization in Tehran. Details are reported previously [17].

2.3. Genetic studies

2.3.1. DNA extraction

DNA was extracted from peripheral blood by using the QIAamp DNA Blood Mini kit (Qiagen, Germany) according to the manufacturer's protocol. Real-time PCR and high resolution melt analysis were performed in the Corbett rotor-gene 6000 instrument (Corbett Research Pty Ltd, Sydney Australia).

2.3.2. High resolution melt analysis

Primers were designed by Beacon Designer 7.91 to flank the genomic regions (PREMIER Biosoft International, USA and were synthesized by TIB MOLBIOL (Germany). Primer sequences are shown in Supplemental Table 1.

Amplicons from all genes were generated under the following conditions used the type-it HRM kit (Qiagen, Germany): one cycle at 95 °C for 15 min; 40 cycles at 95 °C for 15 s, 60.0 °C for 15 s, 72 °C for 15 s; one cycle of 95 °C for 1 s, 72 °C for 90 s and a melt from 70 to 95 °C rising at 0.1 °C per second. The amplification mixture of a total volume of 25 μL included 12.5 μL of HRM PCR master mix, 1.75 μL of 10 μM primer mix, 2 μL of genomic DNA as template and 8.25 μL of RNase-free water. For each genotype reaction, we included sequence-proven major and minor allele homozygote and heterozygote controls. The HRM analysis was performed by instrument software, which allows clustering of the samples into groups based on a difference plot obtained by analyzing the differences in melting curve shape between known controls and samples.

2.4. Statistical analysis

Statistical analysis was performed with SAS 9.3 (Cary, NC) with a nominal level of significance of $P < 0.05$. Pairwise linkage disequilibrium for single nucleotide polymorphism at the same locus was determined using correlation coefficients as described [18]. We used pairwise χ^2 analysis to test for allele frequency differences between HDL-C groups. To test for deviations of genotype frequencies from those predicted by the Hardy–Weinberg equation, we used χ^2 analysis. Logistic regression analysis was conducted to calculate the odds ratio and 95%confidence interval (OR, 95% CI) for risk of depressed HDL-C levels associated with each polymorphism. This analysis was conducted without adjustment, and after adjustment for age, BMI, low physical activity, consumption of saturated fats, and socioeconomic status. In these analyses the reference group consisted of putative low risk genotypes.

3. Results

3.1. Demographic features

This study was conducted in 750 adolescents with mean (SD) age of 14.10 (2.21) years. The mean age and the gender ratio were

[1] Caspian is the name of the world's largest lake, located in Northern, Iran.

not significantly different in the groups with various levels of HDL-C. The general baseline characteristics of the study sample, subdivided into the three subgroups according to HDL-C levels, are presented in Table 1.

3.2. Genetic associations with HDL-C groups defined by plasma levels

Genetic association with HDL-C level was assessed by determining genotype and allele frequencies of each SNP in each subject group (Table 2), and then performing pairwise comparisons of MAFs between groups (Table 3). There was no significant deviation of observed genotype frequencies for any SNP from those predicted by the Hardy–Weinberg equilibrium. Each SNP genotype was assessed individually, since analysis of linkage disequilibrium showed no significant linkage disequilibrium between SNPs that were at individual loci, meaning that all genotypes were essentially independent in this sample (Supplemental Table 2).

The vast majority of pairwise comparisons of MAFs of these genes and SNPs between HDL-C groups was significant (Table 3). Pairwise comparisons between low and high HDL-C groups showed significant between-group differences in MAFs for all SNPs, except for *APOC3* rs5128. Pairwise comparisons between low and intermediate HDL-C groups showed significant between-group differences in MAFs for all SNPs, except for *APOC3* rs5128 and *APOA1* rs2893157. Pairwise comparisons between intermediate and high HDL-C groups showed significant between-group differences in MAFs for all SNPs, except for *ABCA1* *APOC3* rs5128 and *APOA1* rs2893157.

Results of logistic regression analysis for low HDL-C status per risk allele for each SNP are presented in Table 4. It shows that all SNPs increased the risk of depressed HDL-C except than CETP Taq1 that had a protective role. After adjustment for confounding factors, including age, sex, body mass index, low physical activity, consumption of saturated fats, and socioeconomic status, *ABCA1* r1587K and CETP A373P significantly increased the risk of depressed HDL-C, and the protective role of CETP Taq1 remained significant.

4. Discussion

This study, which to the best of our knowledge is the first nationwide study in the pediatric population, revealed several genetic associations with HDL-C levels in Iranian children and adolescents. This study extends the range of human populations in which these associations have been documented. The fact that so many associations were so significant in such a small sample size could be due to the experimental design of this study, or more likely, due to the fact that genetic effects on complex quantitative traits are stronger in the pediatric age group. This is perhaps because in this age group, there has been relatively little cumulative exposure to non-genetic factors, as smoking, obesity, inactivity and poor diet, i.e. variables that can add variability to HDL-C levels.

Previous GWAS investigating the concentrations of HDL-C in other populations identified SNPs at several loci, as associated with HDL-C levels including *ABCA1*, the *APOA1*/*C3*/*A4*/*A5* gene cluster, *CETP*, and *LPL* [3,18]. However, whether these variants also confer risk in populations with a different cultural, geographic and demographic history, such as Iranians, remained unexplored until this study. Thus, we analyzed the associations of plasma HDL-C levels with 10 candidate SNPs, many in coding regions, in 6 biologically important candidate genes in a well-characterized Iranian population. The results show that SNPs in genes encoding LPL, CETP, and *ABCA1*, were independently associated with plasma HDL-C levels. The effects for all associated SNPs were in the same direction as in previous studies. Three genome-wide scans that studied a total of together 8816 individuals identified CETP, LPL, and *ABCA1* as being associated with HDL-C with genome-wide significance [19,20]. Our observations are also in line with the results of a GWAS that showed SNPs in main genes encoding key enzymes in the HDL-C metabolism were independently associated with HDL-C concentrations. Remarkably, for some SNPs in even one gene, as CETP and *ABCA1*, polymorphisms had an independent association with HDL-C concentrations in spite of a strong linkage disequilibrium [21].

Lipoprotein lipase (LPL) is a key enzyme involved in lipolysis of TG-rich lipoproteins [22]. LPL does not act directly on HDL-C, however its action on TG-rich lipoproteins has a significant

Table 1
Characteristics[a] of adolescents in different HDL-C groups: the CASPIAN-III Study.

Characteristics	HDL-C < 5th percentile ($n = 250$)	HDL-C 5–95th percentile ($n = 250$)	HDL-C>95th percentile ($n = 250$)
Age, years	15.0 ± 2.6	14.8 ± 2.4	14.0 ± 2.6
Sex (% female)	48.8	46.4	49.6
Body mass index (kg/m^2)	20.0 ± 4.3	19.0 ± 3.9	18.0 ± 3.4
Systolic blood pressure (mmHg)	105.0 ± 14.3	102.0 ± 12.2	101.0 ± 13.2
Diastolic blood pressure (mmHg)	67.0 ± 10.8	65.3 ± 11.3	65.5 ± 9.5
HDL-C (mmol/L)	26.1 ± 3.1	44.5 ± 0.5	77.5 ± 8.3
Residence area,%			
Urban	27.2	36.6	36.2
Rural	41.6	28.2	30.2

HDL-C, high-density lipoprotein cholesterol.
[a] Data are presented as mean ± SD or percentage.

Table 2
Genotype and allele frequencies of adolescents in different HDL-C groups: the CASPIAN-III Study.

Gene name, polymorphism name and identification number (genotypes and frequencies), minor allele frequency	HDL-C < 5th percentile ($n = 250$)	HDL-C 5–95th percentile ($n = 250$)	HDL-C > 95th percentile ($n = 250$)
LPL D9N rs1801177 (AA/AG/GG)	226/24/0	231/19/0	250/0/0
MAF	0.048	0.038	0
LPL HindIII rs320 (GG/GT/TT)	145/102/3	117/120/13	4/121/125
MAF	0.216	0.292	0.742
LPL S447X rs328 (CC/CG/GG)	228/22/0	203/46/1	163/73/14
MAF	0.044	0.096	0.202
ABCAI V771M rs2066718 (GG/GA/AA)	249/1/0	237/13/0	234/16/0
MAF	0.002	0.026	0.032
ABCAI R1587K rs2230808 (AA/AG/GG)	106/106/38	141/93/16	194/56/0
MAF	0.364	0.250	0.112
*CETP Taq*IB rs708272 (CC/CT/TT)	181/67/2	63/147/40	34/162/54
MAF	0.142	0.454	0.540
CETP A373P rs5880 (CC/CG/GG)	188/58/4	217/33/0	247/3/0
MAF	0.132	0.066	0.006
*APOC3 Sst*I rs5128 (CC/CG/GG)	208/40/2	209/39/2	211/39/0
MAF	0.088	0.086	0.078
*APOA1 Msp*I rs2893157 (GG/GA/AA)	189/59/0	182/63/5	169/73/8
MAF	0.119	0.146	0.178
APOA5 C-1131T rs662799 (CC/CT/TT)	242/3/5	245/2/3	250/0/0
MAF	0.026	0.016	0

MAF, minor allele frequency; HDL-C, high density lipoprotein cholesterol; LPL, gene encoding lipoprotein lipase; *ABCA1*, gene encoding ATP binding cassette transporter A1; CETP, gene encoding cholesteryl ester transfer protein; *APOC3* gene encoding apo C-III; *APOA1*, gene encoding apolipoprotein (apo) A-I; APOA5, gene encoding apo A-V.

Table 3
Allele frequency pairwise comparisons between different HDL-C groups: the CASPIAN-III Study.

	HDL-C < 5th percentile	HDL-C 5–95th percentile
LPL D9N rs1801177		
HDL-C 5–95th percentile	NS (0.44)	
HDL-C>95th percentile	<0.0001	<0.0001
LPL HindIII rs320		
HDL-C 5–95th percentile	0.0058	
HDL-C > 95th percentile	<0.0001	<0.0001
LPL S447X rs328		
HDL-C 5–95th percentile	0.0013	
HDL-C > 95th percentile	<0.0001	<0.0001
ABCAI V771M rs2066718		
HDL-C 5–95th percentile	0.0012	
HDL-C > 95th percentile	0.0002	NS (0.57)
ABCAI R1587K rs2230808		
HDL-C 5–95th percentile	<0.0001	
HDL-C > 95th percentile	<0.0001	<0.0001
*CETP Taq*IB rs708272		
HDL-C 5–95th percentile	<0.0001	
HDL-C > 95th percentile	<0.0001	0.0065
CETP A373P rs5880		
HDL-C 5–95th percentile	0.0005	
HDL-C > 95th percentile	<0.0001	<0.0001
*APOC3 Sst*I rs5128		
HDL-C 5–95th percentile	NS (0.91)	
HDL-C > 95th percentile	NS (0.57)	NS (0.64)
*APOA1 Msp*I rs2893157		
HDL-C 5–95th percentile	NS (0.36)	
HDL-C > 95th percentile	0.022	NS (0.17)
APOA5 C-1131T rs662799		
HDL-C 5–95th percentile	NS (0.27)	
HDL-C > 95th percentile	0.0003	0.0045

HDL-C, high density lipoprotein cholesterol; LPL, gene encoding lipoprotein lipase; *ABCA1*, gene encoding ATP binding cassette transporter A1; CETP, gene encoding cholesteryl ester transfer protein; *APOC3* gene encoding apo C-III; *APOA1*, gene encoding apolipoprotein (apo) A-I; APOA5, gene encoding apo A-V; NS, not significant.

indirect effect on HDL-C metabolism [23]. While LPL gene polymorphisms have been widely associated with variation in plasma TG, our findings are similar to some studies conducted in other populations [24,25]. LPL is also a major candidate gene for ACVD. Meta-analysis supports the protective effect of the *LPL* Ser447Ter variant in both Caucasian and East Asian on ischemic stroke, especially atherosclerotic stroke subtype [26]. An-interesting meta-analysis of 22,734 cases of ACVD and 50,177 controls in 89 association studies also confirmed associations between the rs1801177, rs320, and rs328 polymorphisms and plasma HDL-C and TG, coronary stenosis, and myocardial infarction [27].

CETP is a key plasma protein that affects circulating levels of HDL-C through the transfer of esterified cholesterol from HDL to apo B-containing particles in exchange for TG [28]. The lipid profile usually seen in subjects with CETP deficiency includes elevated HDL-C levels [29]. Most previous studies examining the relationship between CETP variants and plasma HDL-C levels and ACVD risk have investigated the intronic *Taq*IB variant [30,31]. Individuals with the A allele of *Taq*IB SNP are reported to have lower mean CETP activity and higher HDL-C and apo A-I concentrations [30]. In a GWAS, the SNP of CETP had the strongest association with HDL-C of all 948 SNPs genotyped in 122 genes [32]. A meta-analysis of13,677 subjects showed that the CETP *Taq*IB SNP was firmly associated with HDL-C plasma levels and as a result, with the risk of ACVD [33].Our findings are in line with the results of another meta-analysis, which included 39,581 to 68,134 participants for the rs708272 (*Taq*IB) SNP; this underlines the significant influence of the *CETP* gene on HDL-C levels [21]. The association between *Taq*IB polymorphism in the CETP with the serum HDL-C levels has been previously reported on a sub sample of Iranian adults [34]. Another study demonstrated that *Taq*IB SNP might contribute to the genetic risk of developing ACVD in Iran [35]. However, still there is no agreement on the relationship between CETP *Taq*IB SNP and ACVD.

A previous study revealed that the rs5880 allele is associated with higher activity and concentration of the CETP protein and lower HDL-C, were also associated with significantly increased presence of coronary artery calcium and carotid stenosis [36]. Though the minor allele frequency (MAF) of rs5880 is lower, several previous studies showed its significant association with HDL-C. Thus, our results are consistent with several studies that reported significant association of CETP variants on serum HDL-C levels in children [37–41].

ATP binding cassette transporter A1 (*ABCA1*) plays a key role in intracellular cholesterol removal and homeostasis [42]. *ABCA1* transporter has an important physiological role in HDL-C metabolism, so many studies investigated the association of this gene with HDL-C levels. They have confirmed associations between certain SNPs in *ABCA1* and HDL-C concentrations [43–45]. Furthermore, recent GWAS and meta-analysis studies found that SNPs in *ABCA1* were significantly associated with HDL-C [16,18]. A study in Iranian adults showed that R219K SNP could affect HDL-C levels and could influence the risk of atherosclerosis in Iranian population [46]. Another study found that the V771M SNP was associated with higher HDL levels in Turkmen [47]. In a meta-analysis with 6597 cases and 15,369 controls for investigation of the association between the *ABCA1* R219K polymorphism and ACVD risk; the R219K SNP was associated with a higher HDL-C level in Asians and protective for ACVD risk both in Asians and Caucasians. A recent meta-analysis involving 2730 ACVD patients and 2658 controls showed that K allele of the *ABCA1* R219K gene has a protective role for ACVD risk in the Chinese population [48]. A study among Japanese healthy children, documented significant associations of the *ABCA1* K219R and V771M polymorphisms with HDL-C concentrations, and suggested that these alleles have anti-atherogenic effects [49].

APOA1 is part of the *APOA1/C3/C4* gene cluster on human chromosome 11q23 which harbors also *APOA5*. *APOA1* comprises roughly 70% of the HDL-C protein mass, and is an important ligand for HDL binding to cellular receptors, including scavenger receptor class B type 1 and *ABCA1* [50]. Therefore, apo A-I serves as a cofactor for cholesterol esterification and is an important component of the reverse cholesterol transport [51]. The clinical significance of apo A-I is featured by large prospective epidemiological studies have shown that apo A-I levels are strong predictors of ACVD risk [52]. It has also been shown that Apo A-I have a marked protective effect against atherosclerosis in animal models [53].

Apo C-III is a component of TG-rich lipoproteins and HDL-C particles and is transferred to HDL-C during the hydrolysis of TG-

Table 4
Odds ratios for depressed HDL-C status per risk allele for each single nucleotide polymorphism: the CASPIAN-III study.

SNP (rs number)	Unadjusted			Adjusted[a]		
	Or	95% CI for OR	*P*-value	Or	95% CI for OR	*P*-value
APOA1 Msp1	1.87	(1.55, 2.21)	<0.0001	1.01	(0.67, 1.49)	0.98
ABCA1 r1587K	3.16	(2.54, 3.93)	<0.0001	2.43	(1.68, 3.50)	<0.0001
ABCA1 V771M	1.89	(1.62, 2.21)	<0.0001	0.47	(0.19, 1.52)	0.09
CETP Taq1	0.54	(0.42, 0.67)	<0.0001	0.08	(0.05, 0.13)	<0.0001
CETP A373P	2.46	(2.08, 2.92)	<0.0001	5.79	(3.40, 9.84)	<0.0001
APOC3 Sst1	2.02	(1.71, 2.38)	<0.0001	1.08	(0.68, 1.72)	0.73

HDL-C, high density lipoprotein cholesterol; SNP, single nucleotide polymorphism; OR, Odds ratio.
[a] The model adjusted for age, sex, body mass index, low physical activity, consumption of saturated fats, and socioeconomic status.

rich lipoproteins. The main physiological role of Apo C-III is inhibitory effect on LPL [54]. For the *APOC3* gene, data on the influence of genetic variability on HDL-C levels are heterogeneous and seems to be weak, which is consistent with the observations in this study.

*APOA*5 is within to the *APOA1*/*C*3/*A*4/*A*5 gene cluster. The human *APOA*5 gene consists of four exons and codes a 369 amino acid protein, expressed only in the liver. Apo A-V is predominantly located on TG-rich particles, chylomicrons and VLDL but also on HDL-C, and there is evidence that Apo A-V serves as an activator of LPL. Among the apolipoproteins, *APOA5* seems to have the most pronounced associations with HDL-C levels. Many polymorphisms of *APOA5* were investigated in the literature, and most of them show an association with decreased levels of HDL-C. Only four SNPs are available in HapMap, which are only weakly associated except rs662799 and rs651821 with a strong correlation [55].

It has been shown that APOA5 variant was associated with the severity and progression of coronary atherosclerosis. The minor alleles of *APOC3* −455T > C, APOA5 −1131T > C, and c.553G > T polymorphisms are closely associated with ACSs [56]. The association of the *APOA1*/*C*3/*A*4/*A*5 gene cluster polymorphisms and lipid levels in humans has been evaluated in a large number of studies. However, previous findings are inconsistent [57,58].

In addition to abovementioned studies, our results confirmed the associations of the gene variants and HDL levels in some other studies among adults [59–61]. Given that cholesterol homeostasis often worsens with age, and it may be because of reduction of its physiological functions and the dysfunction in cholesterol efflux [62], the low HDL-C levels of our children may aggravate in future years of their life.

5. Conclusion

The present study show associations that are largely in the same direction as in previous studies on biological effects of the genes in HDL-C biosynthesis, maturation and catabolism and largely supported by the previous data for SNPs in *ABCA1*, *APOA1*, *APOA*5, *CETP* and *LPL* genes.

Assuming that single polymorphisms does not have a firm role as a marker of complex traits, the study of variant combinations provides complementary information that could be clinically more significant. Recent studies on genetic predisposition to dyslipidemia reinforce this idea as they show that certain polymorphism combinations, including variants in genes analyzed in our work, can predict ACVD.

Given our relatively small sample size, the remaining loci and those that did not replicate in this study will require comprehensive investigation using larger samples to exclude or verify their significance in Iranians. Because a healthy lifestyle can help improve HDL-C levels, with a possible benefit on ACVD risk, more attention should be paid to healthy lifestyle from early life and to primordial and primary prevention of chronic non-communicable diseases. Larger genetic studies should be conducted to explore the predisposition to low HDL-C levels and its long-term consequences. Perhaps future GWAS in Iranians should further assist in fine mapping the causal variants in these known loci and reveal novel susceptibility alleles.

Conflict of interest

The authors have no conflict of interest.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.atherosclerosis.2014.08.043.

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