Abstract

**Background:** To investigate the associations of genetic polymorphism with high-density lipoprotein-cholesterol (HDL-C) levels in Iranian adolescents.

**Methods:** This multicentre study was conducted on 10-18 year-old students from 27 provinces in Iran. Logic regression approach was used to determine the main effects and interactions of polymorphisms related to HDL-C levels.

**Results:** The rs708272 polymorphism was significantly related to HDL-C levels. Moreover, rs708272 increased HDL-C levels and had a protective effect on HDL-C. The interaction of rs2230808 and rs5880 polymorphisms as well as the interaction of rs320 and rs708272 polymorphisms were associated with lower HDL-C levels. Furthermore, the interaction of rs320 and rs1801177 polymorphisms was associated with lower HDL-C levels.

**Conclusions:** We found that not only single SNPs, but also interactions of several SNPs affect HDL-C levels. Given the high prevalence of low HDL-C in Middle Eastern populations, further genetic studies are required for detailed analysis.

**Keywords:** Polymorphism, High-Density Lipoprotein, Pediatrics, Logic Regression

1. **Background**

   Non-communicable diseases and notably cardiovascular diseases (CVD) are the primary reason for mortality and the main health burden worldwide (1). Serum lipids and lipoproteins in the pediatric age group are shown to be predictive of future symptomatic CVDs (2). Studies show that lipoproteins, as well as other cardiovascular risk factors, track from childhood to adulthood (3). The assessment of the early onset of CVD risk factors in childhood is important to determine optimal preventive measures, since determining the early development of adult CVDs may help reduce the mortality rate (4, 5). Among lipids, high-density lipoprotein cholesterol (HDL-C) is a protective element against CVD (6). A study presented that having HDL-C 6 to 7 mg/dL higher than average leads to 20% to 27% decrease in the risk of CVD (7).

   Low HDL-C levels are quite prevalent in the Middle-Eastern countries (8). Population-based studies in Iran presented a prevalent low HDL-C among children and adolescents (4, 9, 10). Furthermore, its fifth percentile of Iranian pediatric population is lower than that of European and American population (11, 12). Moreover, it is well documented that HDL-C is affected by both genetic contexts and environmental factors, such as demographics, diet, smoking, and weight disorders (13, 14). Although hormonal, environmental, and social factors could specify HDL-C levels, the genetic component accounts up to 76% of the variation in HDL-C levels (15). Single nucleotide polymorphism (SNP) is the most prevalent genetic discrepancy (16). In the evaluation of genotype data, the effect size of associations between one SNPs and a response is usually small. Therefore, it is assumed that not only single SNPs but also interactions of several SNPs is particularly important (17). Most genetic studies on lipid profiles focused on individual SNPs while SNP-SNP interactions are suggested to have a great effect on the structure of complex diseases. As an example, Kel-
ishadi et al. investigated the genetic association with low concentrations of HDL-C in a pediatric population, (18). In this study, single SNPs were taken into account and the polymorphism of ApoE gene was not included.

To the best of our knowledge, no analysis has been performed to evaluate an association between the interaction of SNPs and low HDL-C in Middle Eastern adolescents. Therefore, the aim of the present investigation was to determine the influence of some polymorphisms (and their interactions) on HDL-C levels performed for the first time on children and adolescents.

2. Methods

2.1. Study Populations

The subjects in this study were randomly determined between individuals in the CASPIAN-III study, Childhood and Adolescence Surveillance and Prevention of Adult Non-communicable disease study. This survey was performed to determine risky behaviors in Iranian school students (2009 - 2010).

The original study included 5528 10 - 18 year-old students selected from Iranian urban and rural regions (19). A total of 734 frozen blood samples were selected.

Low HDL-C levels were determined as concentrations of < 40 mg/dl. Elevated lipid was considered as total cholesterol higher or equal to 200 mg/dl, low-density lipoprotein cholesterol (LDL-C) higher or equal to 130 mg/dL, and triglycerides higher or equal to 150 mg/dL. High waist circumference was determined as WC higher or equal to 90th percentile of the population studied. Elevated fasting blood sugar was defined as FBS higher or equal to 100 mg/dL. Blood pressure higher or equal to 130/85 mmHg was considered as elevated BP (20).

Normal weight Underweight, overweight, and obesity were determined as a BMI Z-score according to world health organization (WHO) definition (21).

Polymorphisms of LPL rs1801177, LPL rs320, LPL rs328, ABCA1 rs2066718, ABCA1 rs22310808, CETP rs708272, CETP rs5880, APOC3 rs5128, APOAI rs2893157, APOA5 rs662799, and ApoE genes related to HDL-C disorder were analyzed (22). Each SNP was represented by the values 0, 1, and 2 based on the pairs of nucleotide. We recoded this variable into 2 binary variables corresponding to a dominant gene (if SNP has at least one variant allele) and a recessive gene (if SNP has 2 variant alleles). Using this method, we generated 2p binary predictors out of p SNPs (23).

2.2. Genetic Studies

2.2.1. DNA Extraction

DNA was extracted from peripheral blood by using the QIAamp DNA Blood Mini kit (Qiagen, Germany). Corbett rotorgene 6000 instruments (Corbett Research Pty Ltd, Sydney Australia) were used for Real-time PCR and high-resolution melt analysis. Primers were produced by Beacon Designer 7.91 to flank the genomic regions (PREMIER Biosoft International, USA, and TIB MOLBIOL (Germany) were used for synthesizing.

The Standard conditions of amplicons production using type-it HRMkit (Qiagen, Germany) were used (24). The sequence-proven major and minor allele homozygote and heterozygote controls were then included. The HRM analysis was performed, and the samples were clustered (24).

2.3. Statistical Analysis

The continuous variables were shown as mean and standard deviation. However, on the other hand, frequencies and percentages were used for categorical variables. The t-test and χ² were used to compare the continuous and categorical variables in boys and girls, when appropriate. The association between polymorphisms and other covariates interactions and HDL-C levels was analyzed using logic regression.

Few approaches were proposed for the direct detection of statistical interactions between SNPs in order to enhance the statistical power of the studies. Logic regression was recently proposed as a generalized regression method to identify interactions between binary predictors associated with a response variable. It can detect complicated interactions between predictors that could play important roles in genetics or discover prognostic factors in medical data. This technique was efficiently applied on SNP data (23).

The form of the logic regression model is as below:

\[
h \left[ E \left( Y \mid X \right) \right] = \beta_0 + \sum_{i=1}^{n} \beta_i X_i + \sum_{j=1}^{m} \beta_j x_j + \sum_{j=1}^{m} Z_j\]

where \(h(\cdot)\) is an appropriate link function for the response and the predictors, e.g. linear \(h \left( E \left[ Y \mid X \right] \right) = E \left[ Y \right]\), and logistic regression \(h \left( E \left[ Y \mid X \right] \right) = \log\left( E \left[ Y \right] / (1 - E \left[ Y \right]) \right)\). \(X\) is the covariate matrix, with \(\beta_i\) as parameters and the \(I_i\) as Boolean combinations of the binary predictors such as \(x_2\) and \(x_5^c\) or \(x_2\), with the operator ‘c’ as complement. Furthermore, the \(Z\) are extra confounders. For each model, a score function was defined reflecting the “quality” of the model under consideration. We endeavored to find Boolean combinations minimizing the scoring function related to the model. Simulated annealing search algorithm was used to search for the best Boolean combinations and estimation of \(\beta_j\) (23).

The association between combination of predictors and low HDL-C level was assessed by the randomization test. The best model was first fitted to the data, the response was permuted in random, and the best model was
re-fitted. This procedure was repeated several times. If the entire scores of the permuted data were considerably worse than that of the original data, the information existed in the predictors; otherwise, there was no connection between the predictors and the response (23).

To avoid over-fitting in logic regression models, the cross-validation test was applied to determine the optimal model size. The data were split into k equal parts. Then, k times, one partition was left as a test set, and for each possible model size, the best model was selected using k-1 out of k parts, after which the score function on the remaining test set part was computed. For each level of model size, the k score function was added and the model size with the smallest overall score function was selected (23).

Logic regression with logit link function was used to recognize the relationship between the input predators and the outcome. We applied R software (version 3.3.0) for analyzing the data.

3. Results

The average age of the adolescents was 14.66 (2.6) years with the male predominance of 51.6% (sex ratio = 1.068). Table 1 represents the summary of demographic and clinical characteristics of the study subjects by gender.

Genotype and SNP allele frequencies were used to assess genetic association with HDL-C levels (Table 2). None of the SNP distributions represented the deviation from Hardy-Weinberg equilibrium.

Figure 1 shows the results from the null model randomization test. The score of the NULL, the best scoring, and that of the randomization models were compared. Accordingly, we can conclude that there is an association between the predictors and the low HDL-C.

Figure 2 displays the result of the cross-validation test. Accordingly, the model with 4 trees and 7 leaves was optimal in terms of the test set deviance and the model size.

Figure 3 displays the ROC curve of fitted model. The Area under the ROC curve was 0.87 (95% CI 0.81, 0.90) indicating the high performance of the proposed method.

Table 3 presents the optimal combination rules for low HDL-C based on the logic regression. The model contains 4 logic combinations significantly affecting the low level of the HDL-C:
Table 1. Characteristics of the Participants

| Variable          | Total (N = 734) | Boys (N = 379) | Girls (N = 355) | P Value |
|-------------------|-----------------|----------------|-----------------|---------|
| Age, y            | 14.66 ± 2.61    | 14.56 ± 2.62   | 14.76 ± 2.59    | 0.30    |
| BMI, kg/m²        | 19.14 ± 4.08    | 19.43 ± 4.20   | 18.82 ± 3.93    | 0.04†   |
| HDL-C, mg/dL      | 49.50 ± 21.93   | 48.88 ± 21.23  | 50.16 ± 22.67   | 0.43    |
| High FBS          | 90 (12.26)      | 35 (9.23)      | 55 (15.49)      | 0.01†   |
| High WC           | 100 (13.62)     | 49 (12.93)     | 51 (14.37)      | 0.57    |
| High TC           | 43 (5.86)       | 21 (5.54)      | 22 (6.2)        | 0.71    |
| High LDL          | 58 (7.90)       | 30 (7.92)      | 28 (7.89)       | 0.99    |
| High TG           | 64 (8.72)       | 28 (7.39)      | 36 (10.14)      | 0.39    |
| Elevated BP       | 23 (3.13)       | 10 (2.64)      | 13 (3.66)       | 0.43    |

Abbreviations: BMI, Body Mass Index; BP, Blood Pressure; FBS, Fasting Blood Sugar; HDL-C, High Density Lipoprotein Cholesterol; LDL, Low Density Lipoprotein Cholesterol; TC, Total Cholesterol; TG, Triglycerides; WC, Waist Circumference.

*Values are expressed as mean ± SD.
†Values are expressed as n (%).
‡Significant values.

The first combination (L₁) contains “((rs2230808 = GG) or (rs5880 ≠ CC))”. This combination indicates that subjects with the GG genotype of ABCA1 rs2230808 or not CC genotype of CETP rs5880 have an odds ratio of 3.65 (95% CI 2.23, 5.98) with low levels of the HDL-C, comparing to the other cases.

The second combination (L₂) is explained by the interaction of LPL rs320 and LPL rs1801177 polymorphisms. The estimated odds ratio for this combination was 2.12 (95% CI 1.4, 3.2), inferring that G allele of LPL rs1801177 and GG genotype of LPL rs320 gene is associated with low HDL-C levels.

The third combination (L₃) contains “((rs320 ≠ TT) or (rs708272 ≠ TT))”. This form suggests that not TT genotype of LPL rs320 or not TT genotype of CETP rs708272 is associated with higher odds of low HDL-C levels. The odds ratio associated with L₃ was 1.55 (95% CI 1.10, 2.17), indicating that, as a group, the subjects who complies with L₃, are estimated to have higher odds of low HDL-C levels compared to the other subjects.

The fourth combination (L₄) was entirely solely by CETP rs708272 polymorphism. The odds ratio related to this combination was 0.071 (95% CI 0.05, 0.1), inferring that T allele of rs708272 polymorphism is related with high HDL-C levels. The deviance as the model score function was 562.64.

4. Discussion

In this cross-sectional multi-center study, we investigated the effect of 11 genetic variants on the low HDL-C levels phenotypes and identified SNPs that were simultaneously associated with the HDL-C levels of adolescents. Although many studies examined the relationship of single SNP and low HDL-C levels, separately, only few studies analyzed interactions of SNPs that associated with low HDL-C levels.

In the analysis of genotype data, the effect size of associations between single SNPs and a response of interest are usually small. Thus it is assumed that not only single SNPs but also interactions of several SNP are effective. The logistic regression is a novel approach for discovering interactions, specifically Boolean combinations of factors that are associated with the response variable (25). There are several methods proposed in the literature to improve the logistic regression such as logic feature selection, (26). It is possible to select important interactions first and to design the final model based upon such predictors. Logic feature selection did not improve the quality of the proposed regression model. However, this method was used as an extension to our regression for low HDL-C level prediction (classification), whose results are shown in the supplementary file, Appendix 1.

Previous studies showed that ApoE gene polymorphisms were associated with CVD and affected the lipid profile. The e4 allele was shown as an independent risk factor for Type 2 diabetes mellitus and cardiovascular disease (27). However, in our study, the relationship between the ApoE carriers and HDL-C levels in adolescents was not significant. In our study, ApoE carriers were significantly dependent with rs708272 (P value <0.01), rs2230808 (P value < 0.05), and rs320 (P value < 0.001) polymorphisms. Such variables were significant in our model. Thus, discarding the ApoE variable would decrease the model redundancy. Meanwhile, an association with ApoE and HDL-C, levels has
been observed in some but not in all studies in the literature (28, 29).

Our findings showed that individuals without CC genotype of CETP rs708272 polymorphism have upper HDL-C levels. In the other words, T allele of rs708272 polymorphism is associated with raised levels of HDL-C. A meta-analysis published in 2003 has systematically shown that CETP rs708272 polymorphism is associated with HDL-C levels (30). The lack of the T allele of rs708272 polymorphism of the CETP gene was related to CAD in the Chinese population only and T allele of the rs708272 was significantly related to higher HDL-C levels in Chinese male (31). The T allele of rs708272 polymorphism was related to elevated plasma HDL-C levels in the Chinese obese population (32). A national study showed that rs708272 polymorphism was protective on dyslipidemia in Iranian children (33). It was shown that the C allele of rs708272 is related to increased CVD and type 2 diabetes mellitus risks (34). In the other study, the relationship between T allele and higher HDL-C levels in Greek children were observed (35). A meta-analysis of 13,677 individuals indicated that the rs708272 polymorphism was highly related to HDL-C concentration and the risk of atherosclerotic CVD, ultimately (36). Furthermore, a significant relation of the T allele of rs708272 polymorphism with high HDL-C levels has been reported for the Framingham (37), Chinese (38), Iranian (39), and Tunisian populations (40).

We observed that an interaction of rs2230808 polymorphism in ABCA1 gene and rs5880 polymorphism in CETP gene is associated with HDL-C levels. Individuals with GG genotype of rs2230808 polymorphism or G allele of rs5880 have lower HDL-C levels comparing to other subjects indicating that both the main effects of this polymorphism and their interactions can affect the HDL-C concentration. The replacement of C by G at amino acid 373 of CETP resulted in the rs5880 polymorphism. The adverse effect of carriers of the rs5880 polymorphism on the HDL-C levels may be explained by increasing plasma CETP concentration (41). The rs5880, CG, and GG genotypes were affiliated with 17.2%, 95.8% lower large HDL-C particle concentrations, and related with 7% and 41% lower HDL-C levels, respectively (42). The G allele of rs5880 was shown to relate to lower HDL-C, while the ischemic CVD risk was unexpectedly related to being decreased 36% in women with the G allele after HDL adjusting (43). A previous study presented that rs5880 polymorphism was negatively associated with lipid profile and resulted in a 4-fold increase in the childhood dyslipidemia risk (33). The multi-ethnic study of Atherosclerosis (MESA) demonstrated that the G allele of rs5880, which is related to higher CETP concentration (19.5%) and activity (9.4%) and lower HDL-C (6.0%), may be explained by increasing plasma CETP concentration (41). The rs5880 polymorphism in the CETP gene is associated with HDL-C levels. Individuals with GG genotype of rs2230808 polymorphism or G allele of rs5880 have lower HDL-C levels comparing to other subjects indicating that both the main effects of this polymorphism and their interactions can affect the HDL-C concentration. The replacement of C by G at amino acid 373 of CETP resulted in the rs5880 polymorphism. The adverse effect of carriers of the rs5880 polymorphism on the HDL-C levels may be explained by increasing plasma CETP concentration (41). The rs5880, CG, and GG genotypes were affiliated with 17.2%, 95.8% lower large HDL-C particle concentrations, and related with 7% and 41% lower HDL-C levels, respectively (42). The G allele of rs5880 was shown to relate to lower HDL-C, while the ischemic CVD risk was unexpectedly related to being decreased 36% in women with the G allele after HDL adjusting (43). A previous study presented that rs5880 polymorphism was negatively associated with lipid profile and resulted in a 4-fold increase in the childhood dyslipidemia risk (33). The multi-ethnic study of Atherosclerosis (MESA) demonstrated that the G allele of rs5880, which is related to higher CETP concentration (19.5%) and activity (9.4%) and lower HDL-C (6.0%), is also related to atherogenic effects (44). Studies of the ABCA1 gene indicated that its both common and rare variants affect levels of HDL-C and the risk of ischemic CVD (45). It was shown that rs2066718 and rs2230808 were related to HDL-C levels (46, 47). Another study in China showed that G allele of rs2230808 was related to decreased HDL-C level (48). The Russian population found that rs2230808 polymorphism did not affect lipids levels in patients with CVD (49). Additionally, in the population of China, no relationship between rs2230808 with lipids levels in patients with type 2 diabetes mellitus was found (50).

Our result showed that an interaction of rs320 polymorphism in LPL gene and rs708272 polymorphism in CETP gene was associated with HDL-C levels. Individuals with TT genotype of rs320 and TT genotype of rs708272 had higher HDL-C levels. The relationship between the rs320, rs801177, and rs328 polymorphisms with both TG and HDL-C, as well as myocardial infarction was analyzed in AVCD

### Table 2. SNP Genotype and Allele Frequencies in the Study Population

| Polymorphism     | Genotype and Allele |
|------------------|---------------------|
| LPL rs1801177 genotypes | AA | AG | GG |
| LPL rs120 genotypes       | GG | GT | TT |
| LPL rs328 genotypes       | CC | CG | GG |
| ABCA1 rs2066718 genotypes | GG | GA | AA |
| CETP rs708272 genotypes   | CC | CT | TT |
| CETP rs5880 genotypes     | CC | CG | GG |
| APOA1 rs2230808 genotypes | AA | AG | GG |
| APOC3 rs3128 genotypes    | CC | CC | GG |
| APOA1 rs1805167 genotypes | GG | GA | AA |
| LPL rs320 genotypes       | AA | AG | GG |
| LPL rs328 genotypes       | CC | CG | GG |
| apoE alleles             | e2 | e3 | e4 |

*ABCAL, gene encoding ATP binding cassette transporter A1; APOA1, gene encoding apo A-I; APOA5, gene encoding apo A-V; APOC3 gene encoding apolipoprotein (apo) C-III; CETP, gene encoding cholesteryl ester transfer protein; LPL, gene encoding lipoprotein lipase.
### Table 3. The Results of the Fitted Logic Regression Model with 4 Boolean Combinations of 7 Binary Predictor Variables to Study Interaction Effects of SNPs and Other Risk Factors on HDL-C

| Boolean Combination | Coefficient | Standard Error | Odds Ratio | 95% Confidence Interval | P Value |
|---------------------|-------------|----------------|------------|------------------------|---------|
| L₁: (rs230808 = GG) or (rs5880 ≠ CC) | 1.295 | 0.251 | 3.65 | (2.23, 5.98) | < 0.0001 |
| L₂: (rs320 = GG) and (rs1801177 ≠ AA) | 0.749 | 0.211 | 2.12 | (1.4, 3.2) | < 0.0001 |
| L₃: (rs320 ≠ TT) or (rs708272 ≠ TT) | 0.436 | 0.17 | 1.55 | (1.04, 2.365) | 0.018 |
| L₄: (rs708272 ≠ CC) | -2.64 | 0.18 | 0.071 | (0.05, 0.1) | < 0.0001 |

AIC 572.64

AUC 0.87

Abbreviations: AIC, Akaike Information Criterion; AUC, Area Under the Curve.

...and control subjects. Moreover, it was observed that carriers of the less common allele of the rs320 polymorphism had 0.04 mmol/L higher HDL-C levels and 0.09 mmol/L lower triglycerides levels than non-carriers (51). The rs320 GG genotype and rs320 G allele were observed to be significantly associated with stroke in Indian population. Also, rs320 GG genotype associated significantly with high levels of TG and low levels of HDL. However, this polymorphism did not show any association with LDL-C and VLDL levels (52). It was reported that in Iranian children and adolescents, carriers of T allele of rs320 polymorphism are associated with lower TG and LDL-C and higher TC and HDL-C (24). Other studies showed that in healthy-weight men with coronary heart disease, the rs320 polymorphism alone might impress the HDL-C concentration, in contrast to rs328 alone, which has no influence on any lipid parameters (53).

Our result showed that an interaction of rs320 polymorphism and rs1801177 polymorphism in in LPL gene was associated with HDL-C levels. Individuals without GG genotype of rs320 or AA genotype of rs1801177 had higher HDL-C levels. The G allele of rs1801177 polymorphism is shown to be associated with lower HDL-C levels and higher LDL-C levels in Iranian adolescents (24). It was reported that rs1801177 polymorphism alone might impress the HDL-C concentration, in contrast to rs328 alone, which has no influence on any lipid parameters (53).

4.1. Study Limitations and Strengths

Our study was a cross sectional. This is in fact one of the limitations of our work. However, the pediatric age group and the region under the study are 2 advantages of our work in comparison with the state-of-the-art.

4.2. Conclusion:

We showed that rs708272 polymorphism in CETP gene has an important effect on the level of HDL-C, independently. Moreover, rs708272 increased HDL-C levels and had a protective effect on HDL-C. The interaction of ABCA1 (rs230808) as well as CETP (rs5880) and the interaction of LPL (rs320) as well as CETP (rs708272) were associated with lower HDL-C levels. Furthermore, the interaction of LPL (rs320) and LPL (rs1801177) was associated with lower HDL-C levels.

Supplementary Material

Supplementary material(s) is available here.

Acknowledgments

The authors would like to thank the entire organization and the large team collaborating with this project. Authors would also like to thank the study participants; they are grateful for their contribution in this study.

References

1. Mathers CD, Loncar D. Projections of global mortality and burden of disease from 2002 to 2030. *PLoS Med.* 2006;3(11):e442. doi: 10.1371/journal.pmed.0030442. [PubMed: 17132052].

2. Frontini MG, Srinivasan SR, Xu J, Tang R, Bond MG, Berenson GS. Usefulness of childhood non-high density lipoprotein cholesterol levels versus other lipoprotein measures in predicting adult subclinical atherosclerosis: the Bogalusa Heart Study. *Pediatrics.* 2008;121(5):924–9. doi: 10.1542/peds.2007-1472. [PubMed: 18450895].

3. Lauer RM, Clarke WR. Use of cholesterol measurements in childhood for the prediction of adult hypercholesterolemia. The Muscatine Study. *JAMA.* 1990;264(23):3034–8. doi: 10.1001/jama.264.23.3034. [PubMed: 2243431].

4. Delavari A, Forouzanfar MH, Alikhani S, Shariﬁan A, Kelishadi R. First nationwide study of the prevalence of the metabolic syndrome and optimal cutoff points of waist circumference in the Middle East: the national survey of risk factors for noncommunicable diseases of Iran. *Diabetes Care.* 2009;32(6):1092–7. doi: 10.2337/dc08-1800. [PubMed: 19279303].
5. Ribas SA, Santana da Silva LC. Anthropometric indices: predictors of dyslipidemia in children and adolescents from north of Brazil. *Nutr Hosp.* 2012;27(4):1228–35. doi: 10.3009/nh.2012.7.4.5798. [PubMed: 23185566].

6. Barter PJ, Caulfield M, Eriksson M, Grundy SM, Kastelein JJ, Koma-jda M, et al. Effects of torcetrapib in patients at high risk for coronary events. *N Engl J Med.* 2007;357(21):2099–22. doi: 10.1056/NEJMoa0706628. [PubMed: 17984165].

7. Gatto AJ, Brinton EA. Assessing low levels of high-density lipoprotein cholesterol as a risk factor in coronary heart disease: a working group report and update. *J Am Coll Cardiol.* 2004;43(5):717–24. doi: 10.1016/j.jacc.2003.08.080. [PubMed: 14998606].

8. Gehani AA, Al-Hinai AT, Zubaid M, Almahmeed W, Hasani MR, Yusu-fali AH, et al. Association of risk factors with acute myocardial infarction in Middle Eastern countries: the INTERHEART Middle East study. *Curr Atheroscler Rep.* 2009;11(4):400–10. doi: 10.1007/s11883-006-0074-0. [PubMed: 16767843].

9. Kelishadi R, Ardalan G, Gheiratmand R, Adeli K, Mehdi Gouya M, et al. Genetic association with low concentrations of high-density lipoprotein cholesterol as a risk factor in coronary artery disease in the population of western Iran. *J Clin Endocrinol Metab.* 2009;94(11):4200–9. doi: 10.1210/jc.2008-4270. [PubMed: 18697860].

10. Gupta N, Goel K, Shah P, Mishra A. Childhood obesity in developing countries: epidemiology, determinants, and prevention. *Endocr Rev.* 2012;33(1):48–70. doi: 10.1210/er.2010-00028. [PubMed: 22240243].

11. Kelishadi R, Ardalan G, Gheiratmand R, Ardalan G, et al. Genetic association with low concentrations of high-density lipoprotein cholesterol as a risk factor in coronary artery disease in the population of western Iran. *J Clin Endocrinol Metab.* 2009;94(11):4200–9. doi: 10.1210/jc.2008-4270. [PubMed: 18697860].

12. Brunham LR, Hayden MR. Human genetics of variation in high-density lipoprotein cholesterol. *Prog Lipid Res.* 2015;58:314–25. doi: 10.1016/j.plipres.2015.01.001. [PubMed: 25603477].

13. Qasim A, Rader DJ. Human genetics of variation in high-density lipoprotein cholesterol. *J Comput Graph Stat.* 2003;12(3):475–511. doi: 10.1198/10618600338222. [PubMed: 26786614].

14. Ahmadzadeh A, Azizi F. Genes associated with low serum high-density lipoprotein cholesterol as a risk factor in coronary heart disease: a working group report and update. *J Am Coll Cardiol.* 2007;49(3):717–24. doi: 10.1016/j.jacc.2006.06.012. [PubMed: 18640641].

15. Kelishadi R, Gheiratmand R, Ardalan G, Adeli K, Mehdi Gouya M, Mohammad Razaghi E, et al. Association of anthropometric indices with cardiovascular disease risk factors among children and adolescents: the CASPIAN-III Study. *Int J Cardiol.* 2007;117(1):40–8. doi: 10.1016/j.ijcard.2006.06.012. [PubMed: 18640641].

16. El-Lebiedy D, Kaslan H, Mohammed AM. Apolipoprotein E gene polymorphism and risk of type 2 diabetes and cardiovascular disease. *Diabetes Res Clin Pract.* 2016;117(2):2826–34. doi: 10.1016/j.diabres.2015.10.004. [PubMed: 26800892].

17. Kavey RE, Daniels SR, Lauer RM, Atkins DL, Hayman LL, Taubert K, et al. American Heart Association guidelines for primary prevention of atherosclerotic cardiovascular disease beginning in childhood. *Circulation.* 2001;103(7):1562–6. doi: 10.1161/hc09910.091059.ch. [PubMed: 11537030].

18. Daniels SR, Greer FR, Committee on N. Lipid screening and cardiovascular health in childhood. *Pediatrics.* 2008;122(2):398–208. doi: 10.1542/peds.2008-1349. [PubMed: 18596007].

19. Boekholdt SM, Thompson JF. Natural genetic variation as a tool in understanding the role of CETP in lipid levels and disease. *J Lipid Res.* 2003;44(6):2080–93. doi: 10.1194/jlr.M000765. [PubMed: 12839975].

20. Lu Y, Tayebi N, Li H, Saha N, Yang H, Heng CK. Association of CETP TaqIB and -629C > A polymorphisms with coronary artery disease and lipid levels in the multi-ethnic Singaporean population. *Lipids Health Dis.* 2013;12:85. doi: 10.1186/1476-511X-12-85. [PubMed: 23758310].

21. Kuczmarski RJ, Ogden CL, Guo SS, Grummer-Strawn LM, Flegal KM, Mei Z, et al. 2000 CDC Growth Charts for the United States: methods and development. *Vital Health Stat 11.* 2002;24:61–90. doi: 10.1016/j.atherosclerosis.2014.08.043. [PubMed: 24904944].

22. Cohen ME, Derossis G, Louizou E, Yannakoulia M, Drenos F, Papsoutsakis C, et al. APOE, CETP and LPL genes show strong association with lipid levels in Greek children. *Nutr Metab Cardiovasc Dis.* 2010;20(1):26–33. doi: 10.1016/j.numecd.2009.02.005. [PubMed: 19401283].

23. Smith JMW, Sacks FM, Jukema JW, Shepherd J, Freeman DJ, McMahon AD, et al. Cholesterol ester transfer protein TaqIB variant, high-density lipoprotein cholesterol levels, cardiovascular risk, and efficacy of pravastatin treatment: individual patient
45. Frikke-Schmidt R. Genetic variation in the ABCA1 gene, HDL cholesterol, and coronary artery disease: the Framingham study. *Atherosclerosis, Thrombosis, and Vascular Biology*. 2000;20(5):223–9. doi: 10.1160/ATVB.20.5.223 [PubMed: 10807749].

46. Frikke-Schmidt R, Nordestgaard BG, Jensen GB, Tybjaerg-Hansen A. Genetic variation in ABC transporter A1 contributes to HDL cholesterol in the general population. *J Clin Invest*. 2004;114(9):1343–53. doi: 10.1172/JCI20361 [PubMed: 15520867].

47. Pajukanta P. Do DNA sequence variations in ABCA1 contribute to HDL cholesterol levels in the general population? *J Clin Invest*. 2004;114(9):1344–7. doi: 10.1172/JCI23466 [PubMed: 15520856].

48. Guo Z, Wu P, Xie D, Wang Q, Liu Y, Cha Z, et al. A new discovered ABCA1 gene polymorphisms and the association of ABCA1 SNPs with coronary artery disease and plasma lipids in Chinese population. *J Med Colleges PLA*. 2016;21(4):179–90. doi: 10.1006/jmed.2016.01.017. [PubMed: 26853140].

49. Kashani Farid MA, Azizi F, Hedayati M, Daneshpour MS, Shamshiri AR, Siasi F. Association between CETP TaqIB and -629 C/A polymorphisms in patients with endogenous hypertriglyceridemia in Chinese population. *Zhonghua Yi Xue Yi Chuan Xue Za Zhi*. 2006;32(6):640–6. doi: 10.1575/j.1749-725X.2006.00084.x [PubMed: 1690943].

50. Wu Y, Bai H, Liu R, Liu Y, Liu BW. [Analysis of cholesterol ester transfer protein gene Taq IB and -629 C/A polymorphisms in patients with endogenous hypertriglyceridemia in Chinese population]. *Zhongguo Yi Xue Yi Chuan Xue Za Zhi*. 2006;23(6):640–6. [PubMed: 1760943].

51. Ordovas JM, Cupples LA, Corella D, Otvos JD, Osgood D, Martinez A, et al. Four polymorphisms of cholesteryl ester transfer protein gene and coronary stenosis in a Tunisian population: a cross sectional study. *Lipids Health Dis*. 2010;9:96. doi: 10.1186/1476-511x-9-96 [PubMed: 20822508].

52. Agerholm-Larsen B, Tybjaerg-Hansen A, Schnohr P, Steffensen R, Nordestgaard BG. Common cholesteryl ester transfer protein mutations of genetic variants in ATP-binding cassette A1 and cholesteryl ester transfer protein and differences in lipoprotein subclasses in the multi-ethnic study of atherosclerosis. *Clin Chem*. 2009;55(5):481–8. doi: 10.1373/clinchem.2008.07995 [PubMed: 19136137].

53. Dergunov AD. Prediction of the influences of missense mutations on cholesteryl ester transfer protein structure. *Arch Biochem Biophys*. 2014;564(6):67–73. doi: 10.1016/j.abb.2014.08.018 [PubMed: 2520589].

54. Tsai MY, Li N, Sharrett AR, Shea S, Jacobs DJ, Tracy R, et al. Associations of genetic variants in ATBP1 and CETP polymorphism with the serum lipid levels in a group of Tehran’s population: an observational study. *Lipids Health Dis*. 2012;13(9):546–53. doi: 10.2459/JCM.0b013e3283569b24 [PubMed: 22854712].

55. Agerholm-Larsen B, Tybjaerg-Hansen A, Schnohr P, Steffensen R, Nordestgaard BG. Common cholesteryl ester transfer protein mutations and increased HDL cholesterol, and possible decreased risk of ischemic heart disease: The Copenhagen City Heart Study. *Circulation*. 2000;102(18):2197–203. doi: 10.1161/01.CIR.102.18.2197 [PubMed: 11056092].

56. Tsai MY, Johnson C, Kao WH, Sharrett AR, Arends VL, Kronmal R, et al. Cholesteryl ester transfer protein genetic polymorphisms, HDL cholesterol, and subclinical cardiovascular disease in the Multi-Ethnic Study of Atherosclerosis. *Atherosclerosis*. 2008;200(2):297–193. doi: 10.1016/j.atherosclerosis.2007.12.038 [PubMed: 1824327].

57. Frikkke-Schmidt R, Nordestgaard BG, Jensen GB, Tybjaerg-Hansen A. Genetic variation in the ABCA1 gene, HDL cholesterol, and risk of ischemic heart disease in the general population. *Atherosclerosis*. 2002;180(2):305–16. doi: 10.1016/j.atherosclerosis.2002.06.005 [PubMed: 1596329].

58. Moghadasi M et al. Association of cholesteryl ester transfer protein-TaqIB polymorphism with the serum lipid levels in a group of Tehran's population: an observational study. *Lipids Health Dis*. 2012;12(1):9:546–53. doi: 10.2459/JCM.0b013e3283569b24 [PubMed: 22854712].

59. Ceja-Espiritu G, Delgado-Enciso I, Ramirez-Flores M, Guzman-Esquivel JA, Brito I, Diaz-Rodriguez LM. The D9N, N291S, and T495G Polymorphisms of the Lipoprotein Lipase Gene Are Not Associated with Homocysteine Levels in Mexican Patients: A Cross-Sectional Study. *Int J Endocrinol Metab*. 2015;13(3):278-87. doi: 10.5812/ijem.2015.00005314.462710. [PubMed: 1555129].

60. Ordovas JM, Cupples LA, Corella D, Otvos JD, Osgood D, Martinez A, et al. Association of cholesteryl ester transfer protein-TaqIB polymorphism with variations in lipoprotein subclasses and coronary heart disease risk: the Framingham study. *Atherosclerosis, Thrombosis, and Vascular Biology*. 2000;20(5):223–9. doi: 10.1160/ATVB.20.5.223 [PubMed: 10807749].

61. Wu Y, Bai H, Liu R, Liu Y, Liu BW. [Analysis of cholesteryl ester transfer protein gene Taq IB and -629 C/A polymorphisms in patients with endogenous hypertriglyceridemia in Chinese population]. *Zhongguo Yi Xue Yi Chuan Xue Za Zhi*. 2006;23(6):640–6. [PubMed: 1760943].

62. Kashani Farid MA, Azizi F, Hedayati M, Daneshpour MS, Shamshiri AR, Siasi F. Association between CETP TaqIB and -629 C/A polymorphisms with the serum lipid levels in a group of Tehran’s population: a cross sectional study. *Lipids Health Dis*. 2010;9:96. doi: 10.1186/1476-511x-9-96 [PubMed: 20822508].

63. Rejeb J, Omezzine A, Boumaiza I, Rejeb N, Nabli N, et al. Four polymorphisms of cholesteryl ester transfer protein gene and coronary stenosis in a Tunisian population. *J Cardiovasc Med (Hagerstown)*. 2012;13(9):546–53. doi: 10.2459/JCM.0b013e3283569b24 [PubMed: 22854712].

64. Dergunov AD. Prediction of the influences of missense mutations on cholesteryl ester transfer protein structure. *Arch Biochem Biophys*. 2014;564(6):67–73. doi: 10.1016/j.abb.2014.08.018 [PubMed: 2520589].

65. Tsai MY, Li N, Sharrett AR, Shea S, Jacobs DJ, Tracy R, et al. Associations of genetic variants in ATBP1 and CETP polymorphism with the serum lipid levels in a group of Tehran’s population: an observational study. *Lipids Health Dis*. 2012;13(9):546–53. doi: 10.2459/JCM.0b013e3283569b24 [PubMed: 22854712].

66. Agerholm-Larsen B, Tybjaerg-Hansen A, Schnohr P, Steffensen R, Nordestgaard BG. Common cholesteryl ester transfer protein mutations, decreased HDL cholesterol, and possible decreased risk of ischemic heart disease: The Copenhagen City Heart Study. *Circulation*. 2000;102(18):2197–203. doi: 10.1161/01.CIR.102.18.2197 [PubMed: 11056092].

67. Tsai MY, Johnson C, Kao WH, Sharrett AR, Arends VL, Kronmal R, et al. Cholesteryl ester transfer protein genetic polymorphisms, HDL cholesterol, and subclinical cardiovascular disease in the Multi-Ethnic Study of Atherosclerosis. *Atherosclerosis*. 2008;200(2):297–193. doi: 10.1016/j.atherosclerosis.2007.12.038 [PubMed: 1824327].

68. Frikkke-Schmidt R, Nordestgaard BG, Jensen GB, Tybjaerg-Hansen A. Genetic variation in the ABCA1 gene, HDL cholesterol, and coronary artery disease: a HuGE association review and meta-analysis. *Am J Epidemiol*. 2008;168(1):123–46. doi: 10.1093/aje/kwn235 [PubMed: 18922999].