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Interaction of cholesterol ester transfer protein polymo-rphisms, body mass index, and birth weight with the risk of dyslipidemia in children and adolescents: the CASPIAN-III study

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ABSTRACT

Objective(s): This study aims to investigate joint association between cholesterol ester transfer protein (CETP) polymorphisms and body mass index (BMI) or birth weight with the risk of dyslipidemia in Iranian children and adolescents.
Materials and Methods: This study was conducted as a sub-study of the "school-based nationwide health survey" (CASPIAN-III). We randomly selected 750 samples from the whole blood samples. Real-time PCR and high resolution melt (HRM) analysis were performed to determine Taq1B (rs708272) and A373P (rs5880) polymorphisms.
Results: Taq1B polymorphism increased HDL-C, and total cholesterol (TC) as well as decreased triglyceride and LDL-C concentrations. LDL-C and triglyceride levels were significantly higher and HDL-C and TC levels were significantly lower among those with A373P polymorphism. CT/TT genotype in Taq1B polymorphism showed a protective effect on dyslipidemia (OR= 0.12, 95%CI: 0.07-0.20). G allele of A373P polymorphism increased the risk of dyslipidemia (OR=4.10, 95%CI: 2.14, 7.83) after adjusting the confounders. We observed interactive effects of CETP gene polymorphisms and BMI or birth weight on dyslipidemia.
Conclusion: Findings showed Taq1B polymorphism might have a protective effect and A373P polymorphism had deleterious effect on dyslipidemia in Iranian children and adolescents. These associations interacted with BMI and birth weight.

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Introduction

Dyslipidemia is one of the serious health problems in all age groups. Both genetic and environmental factors might influence lipid metabolism. The association of childhood excess weight or childhood obesity with dyslipidemia was well documented (1). It is suggested that some metabolic disorders start early in life and can be further agrevated by other factors in the external environment.

The association between birth weight and future health outcomes is still inconsistent. Researchers believe that genetic factors are important determinants of plasma lipid levels in adults; although, it is less clear in children and adolescents. One of the main proteins involved in lipoprotein metabolism is cholesterol ester transfer protein (CETP). CETP is an anti-atherogenic protein in humans and animals. However, according to evidence CETP has atherogenic roles naturally, which function depending on the metabolic settings. Thus, plasma CETP acts as an important protein in the lipid and lipoprotein metabolism and its polymorphisms may affect dyslipidemia and CVD (2). Several single nucleotide polymorphisms (SNP) have been identified within CETP; they influence enzymatic activity or gene expression level. Two common polymorphisms of CETP gene are Taq1B (rs708272) and A373P (rs5880). Taq1B is a polymorphism at 277th nucleotide in the first intron of CETP and has a restriction site for the endonuclease Taq1. Taq1B polymorphism

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may affect the plasma CETP activity and its concentrations, as well as HDL-C levels. A373P polymorphism is a guanine to cytosine mutation at codon 373 in exon 12 of CETP gene that is one of the most common SNPs, found in 1–5 % of Asians and Europeans. It is shown that these polymorphisms are associated with lipid and lipoprotein metabolism in different populations (3).

CETP polymorphisms affect lipid profile levels. However, to our knowledge, no data exist on the interaction of CETP polymorphisms and weight or birth weight and the risk of dyslipidemia in children and adolescents. Given the tracking of atherosclerotic CVD risk factors including dyslipidemia from childhood to adulthood, it is important to determine the predisposing factors of these risk factors in early life. This study aims to investigate joint association between CETP polymorphisms and BMI or birth weight with the risk of dyslipidemia in Iranian children and adolescents.

Materials and Methods

Study population

This study was conducted as a sub-study of the "school-based nationwide health survey", 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.

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. Details of data collection and sampling were published previously (4). For the current study, we randomly selected 750 samples from the whole blood samples kept frozen at -70 ˚C.

The main survey was approved by ethics committees and other relevant national regulatory organizations. The current survey was approved by the Ethical Committee of Isfahan University of Medical Sciences. Written informed consent was obtained from parents and oral assent from children and adolescents.

Physical examination and biochemical measurements

Weight and height were measured under standard protocols using calibrated instruments. BMI was calculated as the weight (kg) divided by the height squared (m^2). World Health Organization (WHO) defines, normal weight as BMI- Z score between -2 and 1, wasting as BMI-Z score between -2 and -3, risk of overweight as BMI-Z score between 1 and 2, overweight as BMI-Z score between 2 and 3, and obesity as BMI-Z score more than 3 (5).

Birth weight was categorized as low birth weight (less than 2500 g), normal (2500–4000 g) and high birth weight (more than 4000 g). Waist circumference was measured at the smallest point of circumference between the iliac crest and the rib cage.

For assessing lipid profile, students were referred to the nearest health center to the school. Fasting venous blood was taken and lipid profile variables including total cholesterol (TC), HDL-C, LDL-C, and triglyceride (TG) were examined. Cut-off points for abnormal levels of lipids included: TC ≥ 200 mg/dl, LDL-C ≥130 mg/dl, HDL-C <35 mg/dl, and TG ≥130 mg/dl (6).

Assessment of physical activity, diet and socioeconomic status

We used the questionnaire of the World Health Organization-Global School-based Student Health Survey (WHO-GSHS) to assess physical activity. To evaluate the pattern of physical activity, three indicators were used including: hours of physical education at school, hours of screening time, and hours spent on sports club training.

To evaluate the family's socioeconomic status (SES), we included questions about the following socioeconomic indicators: (i) parental level of education (illiterate: score 1, less than high school: score 2, high school graduate: score 3, academic education: score 4); (ii) parental occupational status (unemployed: score 1, worker/farmer: score 2, governmental employee: score 3, self-employed: score 4); and (iii) number of household members, and (iv) possessing a family private car (Yes/No). Of note for parental occupational status and level of education, i.e. questions (i) and (ii), only data on the parent with the higher occupational status or education was considered.

For assessing dietary habits, we used questions about the type of bread (i.e. white or whole grain) and the type of fat consumed in meals at home. In addition, students completed a validated food-frequency questionnaire (FFQ). Food items were grouped into the following categories: carbohydrates (rice, bread, pasta, and potato), vegetables (potato and French fries not included), fruit (fresh, dried, juice), dairy products (milk, cheese, yogurt), proteins, including both animal (red meat, poultry, fish, egg) and plant (beans, soy, nuts), fast foods (pizza, hamburgers, sausages, etc.), as well as salty/high fat snacks and sweets/candies.

DNA extraction

DNA was extracted using the QIAamp DNA Blood Mini kit (Qiagen, Germany) according to the manufacturer's protocol from peripheral blood. Real-time PCR and high resolution melt (HRM) analysis were performed in the Corbett rotor-gene 6000 instrument (Corbett Research Pty Ltd, Sydney, Australia). Primers were designed by Beacon Designer 7.91 to flank the

genomic regions (PREMIER Biosoft International, USA) and were synthesized by TIB MOLBIOL (Germany).

Amplicons from all genes were generated under the following conditions using the type-it HRM kit (Qiagen, Germany): one cycle at 95°C for 15 min; 40 cycles at 95 ˚C for 15 sec, 60.0 ˚C for 15 sec, 72 ˚C for 15 sec; one cycle at 95 ˚C for 15 min; 40 cycles at 95 ˚C for 15 sec, 60.0 ˚C for 15 sec, 72 ˚C for 15 sec; one cycle of 95 ˚C for 1 sec, 72 ˚C for 90 sec 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. Primer sequence used for CETP TaqIB rs708272 was F: GTATAGGGATTT-GTGTTTGTCT, R: CCTAACCTGGCTCAGATC and for CETP A373P rs5880 it was F: TCTCCCCAGGATATC-GTGACTAC, R: GCAGCACATACTGGAAATCCAAGA.

Table 1. Characteristics of the study population across the cholesterol ester transfer protein gene polymorphisms: the CASPIAN-III study

	Taq1B			A373P		
	CC	CT+TT	*P*-value	CC	CG+GG	*P*-value
N (%)	278 (37)	472 (62.93)		652 (86.93)	98 (13)	
Boy, %	51.8	51.7	0.97	52.1	49	0.56
Girl, %	48.2	48.3		47.9	51	
Age	14.74±2.62	14.58±2.56	0.44	14.63±2.59	14.70±2.57	0.81
Weight (kg)	47.17±15.45	44.98±13.95	0.06	45.60±14.38	46.95±15.61	0.39
WC (cm)	68.97±12.68	66.63±10.44	0.01	67.47±11.14	67.67±12.81	0.87
FBS (mg/dl)	84.61±14.90	87.61±12.28	0.006	86.66±12.88	85.45±16.27	0.40
SBP (mmHg)	104.02±14.51	102.14±12.65	0.08	102.63±13.13	104.9±14.86	0.33
DBP (mmHg)	66.33±11.07	65.84±10.35	0.56	65.91±10.20	66.74±12.98	0.55
TC (mg/dl)	143.65±35.90	150.31±33.39	0.01	148.89±34.33	141.19±34.67	0.04
LDL-C (mg/dl)	88.41±34.17	78.79±30.97	<0.001	81.38±32.34	88.49±32.94	0.03
HDL-C (mg/dl)	36.03±17.19	57.19±20.52	<0.001	51.79±22.03	33.1±11.47	<0.001
TG (mg/dl)	98.81±47.80	89.69±40.05	0.009	92.49±40.18	96.51±59.88	0.04
Physical activity (%)						
Mild	44.4	30.2		34	44.7	
Moderate	30.9	34.7	0.001	33	35.3	0.04
Vigorous	24.7	35.1		33	20	
BMI (kg/m^2)	19.43±4.28	18.96±3.94	0.14	19.16±4.11	18.92±3.81	0.59
BMI (%)						
Underweight	19	20.8		19.7	22.9	
Normal	61.9	65	0.22	63.6	65.6	0.39
Overweight+ obese	19	14.2		16.6	11.5	
Birth weight (%)						
BW<2500 g	15.2	13.1		13.4	17.0	
BW=2500-4000 g	75.9	78.9	0.65	78.8	70.5	0.17
BW>4000 g	8.9	8.0		7.8	12.5	
Dyslipidemia	70.4	26.6	<0.001	38.2	71.9	<0.001

WC=waist circumference, FBS=fasting blood sugar, DBP=diastolic blood pressure, SBP=systolic blood pressure, LDL-C=low-density lipoprotein, HDL-C=high-density lipoprotein, TG=triglyceride, BMI=body mass index, BW=birth weight

Table 2. Multivariate logistic regression analysis of cholesterol ester transfer protein gene polymorphisms associated with dyslipidemia

	Taq1B			A373P		
	CC	CT+TT		CC	CG+GG	
dyslipidemia		OR (95%CI)	*P*-value		OR (95%CI)	*P*-value
Crude	1	0.15 (0.11, 0.21)	<0.001	1	4.12 (2.57, 6.62)	<0.001
Model 1*	1	0.14 (0.09, 0.22)	<0.001	1	4.31 (2.38, 7.78)	<0.001
Model 2¥	1	0.12 (0.07, 0.20)	<0.001	1	4.10 (2.14, 7.83)	<0.001

*Model 1: adjusted with age, sex, physical activity, BMI, waist circumference
¥Model 2: adjusted with model 1 and fasting blood sugar, diastolic blood pressure, systolic blood pressure

Table 3. Combined association of CETP polymorphisms and BMI or birth weight with dyslipidemia

	TAQ1B			A373P		
	CC	CT+TT		CC	CG+GG	
BMI						
Crude	OR (95%CI)		$P_{interaction}$	OR (95%CI)		$P_{interaction}$
Underweight	1	0.13 (0.06, 0.27)		1	3.27 (1.29, 8.29)	
Normal	1.25 (0.64, 2.43)	0.18 (0.09,0.33)	<0.001	1.26 (0.82, 1.94)	5.65 (2.90, 11.02)	0.02
Overweight+ obese	1.98 (0.82, 4.78)	0.48 (0.22, 1.02)		2.95 (1.71, 5.07)	4.23 (0.95, 4.60)	
Adjusted*						
Underweight	1	0.10 (0.04, 0.24)		1	3.43 (1.20, 9.74)	
Normal	1.14 (0.55, 2.38)	0.16 (0.08, 0.33)	<0.001	1.28 (0.79, 2.09)	5.69 (2.69, 12.02)	0.01
Overweight+ obese	1.67 (0.63, 4.44)	0.49 (0.21, 1.12)		3.17 (1.72, 5.83)	4.36 (0.83, 4.75)	
Birth weight						
Crude	OR (95%CI)		$P_{interaction}$	OR (95%CI)		$P_{interaction}$
BW<2500 g	1	0.09 (0.03, 0.25)		1	2.68 (0.78, 9.12)	
BW=2500-4000 g	0.34 (0.14, 0.86)	0.06 (0.03, 0.16)	<0.001	0.55 (0.34, 0.89)	2.58 (1.27, 5.24)	0.003
BW>4000 g	1.21 (0.27, 5.39)	0.04 (0.01, 0.13)		0.66 (0.32, 1.37)	1.70 (0.46, 6.28)	
Adjusted¶						
BW<2500 g	1	0.22 (0.07, 0.69)		1	4.01 (0.97, 10.48)	
BW=2500-4000 g	0.26 (0.09, 0.74)	0.11 (0.04, 0.27)	<0.001	0.51 (0.30, 0.86)	2.16 (0.98, 4.74)	0.02
BW>4000 g	0.95 (0.18, 4.79)	0.06 (0.02, 0.26)		0.61 (0.27, 1.38)	1.55 (0.38, 6.30)	

CETP= cholesterol ester transfer protein, BMI= body mass index, BW= birth weight
*Adjusted for age, sex, physical activity, waist circumference, birth weight, socioeconomic status, healthy and unhealthy food intake
¶ Adjusted for age, sex, physical activity, waist circumference, BMI, socioeconomic status, healthy and unhealthy food intake

Statistical analysis

The statistical analyses were performed using Statistical Package for the Social Sciences (ver 20.0) software (Chicago, IL, USA). The data for continuous variables is expressed as mean±SD. The Student's t-test or Mann-Whitney test, as appropriate, were used to determine differences in continuous variables. Categorical variables are presented as percentage. The Pearson's Chi-square test or Fisher's exact test, as appropriate, were used to determine the differences in categorical variables. The multivariate logistic regression analyses was performed to determine how the association of CETP gene polymorphisms with the risk of dyslipidemia after controlling for blocks of covariates related to patient demographics (age, sex), anthropometric characteristics (BMI and waist circumference), and the patient's usual physical characteristics (e.g., physical activity, fasting blood sugar, diastolic blood pressure, and systolic blood pressure). Exploratory interaction analyses based on CETP polymorphisms and BMI/birth weight were also run using Generalized Linear Model, with and without confounder adjustment (age, sex, physical activity, waist circumference, socioeconomic status, healthy, and unhealthy food intake). Interactions were examined by adding product terms to the models. A threshold of *P*-values ≤0.05 was used to declare statistical significance.

Results

Characteristics of study participants according to CETP polymorphisms are presented in Table 1. *P*-value of Hardy-Weinberg expectations was 0.073 for Taq1B polymorphism and 0.75 for A373P polymorphism.

The multivariate logistic regression analysis showed a protective effect of CT/TT genotype on dyslipidemia in the crude and adjusted models. G allele of A373P polymorphism increased the risk of dyslipidemia with an OR of 4.12 (95% CI: 2.57- 6.62, *P*-value <0.001) in the crude and adjusted models (Table 2).

We evaluated the joint effect of the Taq1B polymorphism or A373P polymorphism and BMI for the risk of dyslipidemia (Table 3). We observed interactive effects of CETP gene polymorphisms and BMI on dyslipidemia (*P-interaction* < 0.05). On joint analysis, combination of carrying the T allele with BMI was inversely associated with dyslipidemia. In the crude analysis, we observed a decreased risk of dyslipidemia for the subjects with CT/TT genotypes of

the Taq1B polymorphism as well as persons with a BMI in underweight or normal categories, with an OR of 0.13 (95% CI: 0.06-0.27) and 0.18 (95% CI: 0.09-0.33), respectively. Adjustment for the covariates did not change the statistical significance (*P*-value<0.05). Among individuals with a BMI in underweight or normal categories, carrying CG/GG was positively associated with dyslipidemia and carrying the G allele increased the risk of dyslipidemia (OR=3.27, 95% CI: 1.29-8.29 and OR=5.65, 95% CI: 2.90, 11.02, respectively). Adjustment for the covariates did not change the statistical significance (*P*-value<0.05).

The results for the joint effects of the Taq1B polymorphism or A373P polymorphism with birth weight on dyslipidemia and P-interaction are presented in Table 3. We observed interactive effects of CETP gene polymorphisms and birth weight on dyslipidemia (*P-interaction* < 0.05). In crude and adjusted analysis, exposure to both the CT/TT genotypes for the Taq1B polymorphism and the birth weight in each category posed a decreased risk for the dyslipidemia (*P*-value< 0.05). In the crude analysis, among persons with normal birth weight, carrying CG/GG for A373P polymorphism was positively associated with dyslipidemia and carrying the G allele increased the risk of dyslipidemia (OR=2.58, 95% CI: 1.27, 5.24). This association was no more significant after adjustment for the covariates.

Discussion

Although the risk of dyslipidemia in younger populations has been increasing over the last few decades, much less effort has been made in understanding the gene-environmental interaction in lipid metabolism in younger populations, especially in the young Iranian population. The additive effect of the interaction between genetic and environmental factors is greater than the contribution of either risk factor (7).

The present study suggested the association between the CETP polymorphisms and dyslipidemia in Iranian children and adolescents. Taq1B polymorphism had protective effect on dyslipidemia. However, A373P polymorphism had adverse effect on dyslipidemia. Furthermore, combined effects were observed between the CETP polymorphisms and BMI or birth weight on the risk of dyslipidemia. Genome wide association studies (GWAS) identified strong associations between CETP and plasma lipid concentrations in adults (8). However, the role of genetic factors in the plasma lipid levels is less clear in children (2).

It is not clear whether the main function of CETP is proatherogenic or antiatherogenic. There is still a huge controversy over the association of Taq1B polymorphism with risk of CVD. Our results showed Taq1B polymorphism improved serum lipid levels and decreased the risk of dyslipidemia. Although various previous studies (9, 10) have shown a lower CETP activity, higher HDL-C levels, lower postprandial triglyceride, lower risk of CVD and atheroprotective effect in T allele carriers in Taq1B polymorphism. Others suggested that the T allele had no protective effect or even increased CVD risk in adults (11, 12). In addition, the association of the T allele with higher HDL-C levels was observed in children (2).

The mechanism by which the CETP polymorphisms influence lipid profiles is still not understood. Taq1B polymorphism changes the activity of CETP and affects HDL-C concentrations and probably does not affect protein folding. This association may be population specific and is influenced by environmental factors, such as BMI (13).

Our findings showed the A373P polymorphism had deleterious effects on lipid profile levels and approximately increased fourfold the risk of dyslipidemia in children and adolescents. Agerholm-Larsen *et al* (14) reported the A373P polymorphism in CETP associated with decrease in HDL-C levels in both genders. Two studies (15, 16) found A373P polymorphisms were associated with increasing relative risk of subclinical cardiovascular disease (relative risk = 1.22, p = 0.018) and 12.2% higher triglyceride concentration (*P* = 0.03). They showed that the A373P polymorphism was associated with higher CETP activity and concentration, lower HDL-C levels and atherogenic effects. The Copenhagen City Heart Study reported that A373P polymorphism reduced levels of HDL-C (14). However, when 1,236 French and Irish subjects were examined, there was no change in CETP activity and HDL-C levels in A373P polymorphism (17).

The substitution of C for G at amino acid 373 leads to the A373P polymorphism and increases in mass. Adverse effect of the A373P polymorphism can be explained by increasing plasma CETP activity and its concentration. Effect of this SNP on serum lipids is due to chance or indicates a functional effect on CETP gene expression and needs to be assessed by further studies (18). Gene-environment interactions are common in the pathogenesis of prevalent complex disorders such as dyslipidemia. In our study, the SNPs as well as BMI or birth weight were associated with dyslipidemia. Combined association analyses showed that the Taq1B polymorphism had a protective effect and the A373P polymorphism had an adverse effect on dyslipidemia only in underweight and normal weight subjects in both crude and adjusted models. Findings showed the relationship between the Taq1B polymorphism and HDL-C levels was modified by environmental factors such as obesity (19). It is reported that the association between Taq1B genotype and HDL-C levels was attenuated by obesity (20). Another study confirmed these findings (21). However, Vohl *et al* (22) showed Taq1B polymorphism was not associated with higher HDL-C levels in men with hyperinsulinemia and obesity.

In this study, we observed that dyslipidemia was not associated with the Taq1B or A373P polymorphisms in obese subjects. This has been supported by others who found no association between some lipid profiles such as HDL-C concentrations and Taq1B among subjects in the highest BMI tertile (26.2–40.4 kg/m^2). However, they reported this association in the lowest BMI tertile similar to our findings (20, 23). Study showed that obesity was able to decrease the protective cardiovascular effect of the T allele in Taq1B polymorphism (24).

Findings showed higher CETP activity in obese subjects significantly in comparison with controls (25). A similar finding was reported among obese children (26).

Our findings showed that Taq1B polymorphism had a protective effect on dyslipidemia in all categories of birth weight, and the A373P polymorphism only in normal birth weight category had a significantly adverse effect on dyslipidemia. According to previous studies low birth weight correlated with higher risk for metabolic syndrome and lipid metabolism disorders in young adults. Impaired growth in utero may relate to the development of lipid disorders. However, the evidence on the correlation between birth weight and the lipid levels is less in agreement (27). British birth cohort showed inverse associations between birth weight and total cholesterol, LDL-C and triglyceride levels (28). Researchers supposed genetic factors and their interactions could potentially affect associations between birth weight and lipid levels (28, 29).

The mechanisms related to birth weight and lipid levels are still not fully understood. Intrauterine environment, including nutritional status and fetal exposure to stress may lead to impaired fetal growth, structural changes in the liver and permanent changes in lipid metabolism (27).

The main limitation of this study is the cross-sectional nature of the associations. The strengths of the present study are its novelty in the pediatric age group and a relatively large number of population-based samples.

Conclusion

The present study showed that Taq1B polymorphism had a protective effect and A373P polymorphism had a deleterious effect on dyslipidemia in Iranian children and adolescents. These associations interact with BMI and birth weight. More investigation is needed to assess the gene-environment interaction in children and adolescents.

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