Impact of variants in CETP and apo AI genes on serum HDL cholesterol levels in men and women from the Polish population

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Submitted: 29 June 2015
Accepted: 3 November 2015

Arch Med Sci 2016; 12, 6: 1188–1198
DOI: 10.5114/aoms.2016.60870

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Abstract

Introduction: Polymorphisms in the cholesterol ester transfer protein (CETP) gene and apolipoprotein AI (apo AI) gene are identified as the most common genetic factors influencing high-density lipoprotein cholesterol (HDL cholesterol) levels. Low HDL cholesterol is an important risk factor for cardiovascular disease. We investigated the effect of the TaqIB polymorphism of the CETP gene and the 75G/A polymorphism of the apo AI gene on the HDL cholesterol concentration in a sample of Polish adults.

Material and methods: A total of 621 subjects, 414 women and 207 men, were included in this study. Lipid levels were measured using standard protocols, and apolipoprotein AI was determined by immunoturbidimetric assay. CETP and apo AI genotyping was performed using a restriction fragment length polymorphism based method.

Results: Significantly lower HDL cholesterol concentrations were found in B1B1 homozygotes than in carriers of the B2 allele of the TaqIB polymorphism in the CETP gene among both men and women. In GG homozygotes of the 75G/A polymorphism in the apo AI gene lower HDL cholesterol levels were observed, but the difference did not reach statistical significance. A statistically significant association of low HDL cholesterol (< 25th percentile) with CETP genotypes was found in women ($p < 0.0001$) and in men ($p = 0.0368$).

Conclusions: These data demonstrate a significant impact of the TaqIB polymorphism in the CETP gene on HDL cholesterol levels in the studied Polish population, while the effect of the 75G/A polymorphism in the apo AI gene appears not to be significant.

Key words: high-density lipoprotein cholesterol, cholesterol ester transfer protein, apolipoprotein AI, gene polymorphism.

Introduction

The role of lipoproteins in pathogenesis of atherosclerosis and the association of lipid profile with the risk of cardiovascular disease (CVD) and myocardial infarction (MI) is well accepted [1, 2]. In clinical practice CVD risk assessment is based on a positive association of low-density lipoprotein cholesterol (LDL cholesterol) and an inverse association of high-density lipoprotein cholesterol (HDL cholesterol) with disease development

and occurrence of clinical events [3, 4]. Nowadays, however, despite recognition of the atheroprotective properties of high-density lipoproteins, the influence of a rise in HDL cholesterol on CVD and MI risk is questioned [5–7]. There is no doubt that the relation between HDL and cardiovascular risk depends on the functional properties of HDL particles, and plasma HDL cholesterol concentration is not a marker of HDL function but a result of various processes of the complex metabolism of HDL particles [8]. However, measurement of HDL cholesterol concentration is recommended for assessment of CVD risk and efficiency of hyperlipoproteinemia treatment [9]. Therefore, to improve our understanding of lower and increased HDL cholesterol levels, new data are needed, which on one hand will describe the association between different HDL functions and HDL cholesterol concentrations, and on the other hand will enhance our knowledge on the impact of both genetic and environmental factors on HDL cholesterol in different populations.

Various genetic loci have been identified to affect plasma HDL cholesterol concentrations. However, some genome-wide association studies have suggested that the cholesterol ester transfer protein (CETP) locus with the TaqIB polymorphism is much more significant than any other locus correlated with HDL cholesterol levels [10, 11]. A meta-analysis also revealed an association of the 75G/A polymorphism in the apolipoprotein AI gene (apo AI) with an increase in plasma apo AI levels, but mainly in men [12]. In addition, environmental factors can significantly influence HDL metabolism and reduce or enhance plasma HDL cholesterol concentrations [13]. An interaction between obesity and genetic factors related to HDL cholesterol levels is also of importance [14]. Deficiency of CETP caused by mutations in the CETP gene is characterized by increased HDL cholesterol, but its relation to CVD risk is not clear [15]. Anti-atherogenic and pro-atherogenic activity of CETP in plasma has been considered. CETP activity influences reverse cholesterol transport and also a pool of lipoproteins known to be associated with atherosclerosis. So far, CETP inhibitors besides increasing HDL cholesterol have not been found to be associated with cardiovascular benefit [16, 17]. Although large epidemiological studies have been conducted, the role of CETP in the development of atherosclerosis is still unclear. CETP polymorphisms associated with reduced CETP mass and activity were related to a decrease in cardiovascular risk [18–20]. On the other hand, the same genetic variants which showed reduced CETP levels and increased HDL cholesterol levels have been shown to be associated with increased coronary mortality in statin-treated coronary artery disease patients [21].

The aim of the present study was to assess the frequency of CETP TaqIB and 75G/A apo AI genetic variants and their effects on serum HDL cholesterol concentrations in Polish men and women as well as the influence of the interaction between these genetic variants, obesity and hypertriglyceridemia on HDL cholesterol.

Material and methods

Study population and clinical investigation

For the study 621 unrelated adults of Polish nationality were consecutively recruited on the basis of clinical investigation from subjects who had been directed between September 2011 and December 2014 to the Outpatient Clinic at the National Food and Nutrition Institute in Warsaw for routine general health screening or due to obesity. All participants underwent a through medical evaluation including complete health history and physical examination. Data on blood pressure, height, weight, smoking and medical history were collected. The recruited subjects fulfilled the following criteria: had no signs or symptoms of thyroid or other endocrine diseases, renal and hepatic disorders, were-non diabetic, had no history of alcoholism, myocardial infarction or stroke, their body mass index (BMI) was < 35 kg/m^2, and within the last 3 months before the study were not receiving medications known to influence plasma lipid levels (and women did not use hormonal therapy) as well as anti-inflammatory drugs. Postmenopausal status was defined as age at least 55 years and no natural menses at least 6 months before study entry. Increased serum lipid levels, BMI in the range 25–34 kg/m^2, and hypertension did not exclude subjects from the study. Written informed consent was obtained from each individual before participation in the study. The study protocol was approved by the Ethics Committee at the National Food and Nutrition Institute in Warsaw and was in accordance with the principles of the Declaration of Helsinki.

Blood was collected after overnight fasting. Concentrations of serum total cholesterol, HDL cholesterol and triglycerides (TG) were determined using routine laboratory methods on a biochemical analyzer. The LDL cholesterol levels were calculate using the Friedewald formula. In the serum samples the levels of apo AI were measured using monoclonal antibodies against apo AI (Pointe Scientific) by the immunoturbidimetric method.

DNA genotyping

DNA was extracted from the leukocytes of the whole blood by the DNA isolation kit (A&A Biotechnology, Poland). The TaqIB polymorphism (rs708272) in intron 1 of the CETP gene and

75G/A polymorphism (rs670) of the apo AI gene were detected by a polymerase chain reaction (PCR)-based method using primers described previously [22, 23]. For the CETP TaqIB polymorphism the amplification mixture included 25 pmol of each primer, 100 ng of genomic DNA, 0.2 mmol/l of each dNTP, 1.5 mM $MgCl_2$, and 0.2 U Taq polymerase (BioTaq, Bioline Reagents, UK) in a total volume of 25 µl. Amplification was performed for 35 cycles of 1 min at 94°C, 30 s at 64°C, and 1.5 min at 72°C with an initial denaturation period of 4 min. 20 µl of PCR products was digested with the restriction enzyme *TaqI* according to the recommendations of the supplier (Roche Diagnostics GmbH, Germany). Fragments were separated on 2.5% agarose gel (SeaKem LE Agarose, Lonza, USA) and stained with ethidium bromide. One fragment of 505 bp indicated the absence of the TaqI restriction site (B2B2 genotype), two fragments of 415 and 90 bp indicated the presence of the restriction site (B1B1), and three fragments of 505, 415, and 90 bp indicated heterozygosity for the restriction site (B1B2).

For the apo AI 75G/A polymorphism 25 µl of reaction volume contained 50 ng of genomic DNA, a PCR buffer with 1.5 mM $MgCl_2$, 0.01 mM dNTP, 100 µM of both primers, and 1 U of Taq DNA polymerase (Fermentas, Lithuania). Amplification conditions used with the gradient thermal cycler (MJ Research, USA) were as follows: an initial incubation at 94°C for 5 min, followed by 35 cycles of incubation at 94°C for 1 min, 60°C for 30 s and 72°C for 30 s, with a final extension at 72°C for 5 min. The PCR product was digested overnight with 3 U of the *MspI* enzyme (Roche Diagnostics GmbH, Germany); digested fragments were separated on 3% agarose gel, stained with ethidium bromide, and visualized under UV light. Fragments of 209 and 179 bp indicated the absence of the *MspI* restriction site (AA genotype), fragments of 209 and 113 bp indicated the presence of the restriction site (GG), and fragments of 209, 179 and 113 bp indicated heterozygosity for the restriction site (GA).

Statistical analysis

Data were presented as mean and SD. Ranges of HDL cholesterol and apolipoprotein AI concentrations were obtained as the 25th and 75th percentile determined separately for women and men. Allele frequencies were assessed by the gene-counting method. Chi-square (χ^2) analysis was used to estimate the Hardy-Weinberg equilibrium and to compare genotypic and allelic frequencies among the study groups. One-way ANOVA was used in the analysis between groups which were normally distributed while the Kruskal-Wallis test was used in the absence of normal distribution. Qualitative variables were coded as 0-1 dummy variables. Odds ratios (ORs) (crude and adjusted for confounding factors) were calculated for low HDL (< 25th percentile) and high HDL (> 75th percentile), and among both genders. The following confounding factors were taken into account: smoking, obesity, menopause, hypertriglyceridemia (TG > 200/dl), hypercholesterolemia (total cholesterol ≥ 200 mg/dl), apolipoprotein AI < 25th percentile, 75G/A and CETP gene polymorphism. Of them, CETP gene polymorphism, obesity, TG > 200 mg/dl and Apo AI < 25th percentile were statistically significant (crude analysis) and thus remained in the adjusted regression model. Adjusted logistic regression was used to test whether the effect of B1B1 genotype of CETP gene polymorphism influenced the incidence of low HDL cholesterol (< 25th percentile) in the presence of confounders (obesity, TG > 200 mg/dl and Apo AI < 25th percentile). Separately, adjusted logistic regression was used to confirm the effect of the B2 allele of the CETP polymorphism on incidence of high HDL cholesterol (> 75th percentile) in the presence of confounders (BMI < 30 kg/m^2, TG ≤ 200 mg/dl and Apo AI > 75th percentile).

Results from the logistic regression model are presented as ORs with 95% confidence intervals (CIs). A two-tailed *p*-value of $p < 0.05$ was considered statistically significant. The Statistica software package version 10 was used for the statistical analysis.

Results

Table I provides a summary of the demographic and biochemical characteristics of the study participants: 621 subjects, 207 (33%) men and 414 (67%) women.

The frequencies of genotypes and alleles of the 75G/A polymorphism in the apo AI gene and the TaqIB polymorphism in the CETP gene in the studied group are presented in Table II. The distributions of genotypes of both variants did not deviate from Hardy-Weinberg equilibrium ($p > 0.05$). There was no statistically significant difference in the frequency of alleles and genotypes according to gender, BMI, smoking or menopausal status (data not shown). In additional statistical analysis, no significant difference in frequency of GG homozygosity in the 75G/A polymorphism among women with the B2 allele as compared to women with the B1B1 genotype was found ($p = 0.481$).

No significant differences in lipids, lipoproteins and apolipoprotein AI levels between GG homozygotes and carriers of the A allele of the 75G/A polymorphism in the apo AI gene were observed among both men and women (Table III). However, among women GG homozygotes were characterized by about 11% lower apo AI concen-

Table I. Demographic and biochemical characteristics of the study group

Parameter	All subjects	Women	Men
N (%)	621	414 (67)	207 (33)
Age [years]	46 ±14	46 ±13	46 ±14
Height [cm]	168 ±19	163 ±6	178 ±7*
Weight [kg]	75 ±16	70 ±13	87 ±15*
BMI [kg/m^2]	27 ±4	26 ±5	28 ±4*
SBP [mm Hg]	124 ±15	122 ±15	129 ±13*
DBP [mm Hg]	78 ±10	76 ±10	82 ±10*
Triglycerides [mg/dl]	116 ±61	104 ±50	141 ±72*
Total cholesterol [mg/d]	196 ±36	197 ±35	195 ±36
HDL cholesterol [mg/dl]	56 ±14	60 ±14	47 ±10*
LDL cholesterol [mg/dl]	116 ±33	115 ±32	119 ±33
Apolipoprotein AI [mg/dl]	152 ±33	157 ±33	140 ±28*
Glucose [mg/dl]	86 ±12	85 ±12	91 ±12*
HDL-C/Apo AI	0.3786 ±0.0814	0.3882 ±0.0808	0.3587 ±0.0794*
TG/HDL-C	2.3087 ±1.754	3.126 ±2.094	1.899 ±1.389*
AIP	–0.0956 ±0.2589	–0.1521 ±0.242	0.0223 ±0.254*

*Data are presented as mean ± SD. SBP, DBP – systolic and diastolic blood pressure. AIP (Atherogenic Index of Plasma) was calculated according to the following formula: AIP = log (TG mmol)/(HDL mmol). Ratio TG/HDL-C calculated in mg/ml. *p < 0.05 between men and women, ANOVA or Kruskal-Wallis test was used.*

Table II. CETP and apo AI genotype distribution and allele frequencies in studied group

Genotype	All subjects, *n* (%) *N* = 621	Women, *n* (%) *N* = 414	Men, *n* (%) *N* = 207
CETP:			
B1B1	220 (35)	139 (33)	81 (39)
B1B2	303 (49)	202 (49)	101 (49)
B2B2	98 (16)	73 (18)	25 (12)
Frequency of the B2 allele	0.402	0.420	0.365
$\chi^2 = 0.1401$; $p = 0.708$			
Apo AI:			
GG	376 (61)	247 (60)	129 (63)
GA	201 (33)	133 (33)	68 (33)
AA	38 (6)	30 (7)	8 (4)
Frequency of the A allele	0.225	0.235	0.205
$\chi^2 = 2.476$; $p = 0.1155$			

Pearson's χ^2 analysis was used.

trations as compared to A allele carriers. 71% of studied women were premenopausal and 29% were postmenopausal. No significant difference in HDL cholesterol between women before and after menopause was found among GG subjects or among A allele carriers (data not shown). Significantly lower HDL cholesterol concentrations were found in B1B1 homozygotes than in carriers of the B2 allele of the TaqIB polymorphism in the CETP gene among both men and women (Table IV). These differences were not accompanied by any significant differences in serum triglycerides, total cholesterol or apo AI concentrations. In further statistical analyses (data not shown) the significant effect of the TaqIB polymorphism in the CETP gene on HDL cholesterol was observed in both premenopausal ($p = 0.003$) and postmenopausal women ($p = 0.008$). The HDL/apo AI ratio was higher in female B2 allele carriers compared to female B1B1 homozygotes ($p = 0.0422$), while in male study participants no statistically significant difference was found (Table IV).

It is well known that HDL metabolism is related to the metabolism of triglyceride-rich lipoproteins and lower serum HDL cholesterol concentrations occur in subjects with hypertriglyceridemia [9, 24]. In the whole group 9% of subjects were hypertriglyceridemic (serum TG > 200 mg/dl, according to [25]), 5% among women and 17% among men. The ratio between serum triglycerides and HDL cholesterol was calculated and presented as TG/HDL-C (concentrations expressed in mg/dl) and also as the atherogenic index of plasma (AIP) calculated according to the following formula AIP = log (TG)/(HDL), where both serum triglycerides and HDL cholesterol concentrations were expressed in molar concentrations [26]. In the studied males significantly higher AIP was observed in B1B1 homozygotes compared to B2 allele carriers ($p = 0.0412$), while no differences were noted in women (Table IV). The commonly used TG/HDL ratio was significantly higher in B1B1 homozygotes compared to B2 allele carriers ($p = 0.0348$ among women and $p = 0.0258$ among men). No association between 75G/A polymorphism of the apo AI gene and AIP or TG/HDL was found (Table III).

We analyzed the association of CETP TaqIB variants and apo AI-75G/A variants with HDL cholesterol concentrations lower than the 25th percentile, between the 25th and 75th, and higher than the 75th percentile of the HDL cholesterol range observed, separately in studied men and women. For CETP TaqIB variants a significant association

Table III. Association of 75G/A polymorphism of apo AI gene with lipid profile

Parameter	Women *N* = 414		Men *N* = 207	
	A allele carriers (GA + AA) *n* = 163	GG *n* = 247	A allele carriers (GA + AA) *n* = 76	GG *n* = 129
Age [years]	47 ±14	45 ±13	46 ±14	46 ±13
BMI [kg/m^2]	26 ±5	26 ±5	27 ±4	28 ±4
SBP [mm Hg]	122 ±15	121 ±14	131 ±13	128 ±13
DBP [mm Hg]	76 ±11	76 ±10	83 ±10	82 ±10
Triglycerides [mg/dl]	105 ±54	103 ±48	144 ±78	136 ±67
Total cholesterol [mg/dl]	198 ±36	196 ±35	196 ±39	193 ±34
HDL cholesterol [mg/dl]	62 ±14	59 ±14	48 ±11	47 ±10
LDL cholesterol [mg/dl]	115 ±33	115 ±32	119 ±35	119 ±31
Apolipoprotein AI [mg/dl]	162 ±34	155 ±33	144 ±28	138 ±29
HDL-C/Apo AI	0.392 ±0.079	0.386 ±0.082	0.360 ±0.074	0.358 ±0.083
TG/HDL-C	1.904 ±1.577	1.899 ±1.264	3.294 ±2.296	2.986 ±1.950
AIP	–0.164 ±0.144	0.237 ±0.245	0.009 ±0.267	0.0292 ±0.249

Data are presented as mean ± SD. SBP, DBP – systolic and diastolic blood pressure. AIP (Atherogenic Index of Plasma) was calculated according to the following formula: AIP = log (TG mmol)/(HDL mmol). Ratio TG/HDL-C calculated in mg/ml. Differences between A allele carriers and GG homozygotes were not statistically significant; ANOVA or Kruskal-Wallis test was used.

Table IV. Summary for the biochemical parameters in different variants of CETP-TaqI polymorphisms

Parameter	Women *N* = 414		Men *N* = 207	
	B1B1 *n* = 139	B2 allele carriers (B1B2 + B2B2) *n* = 275	B1B1 *n* = 81	B2 allele carriers (B1B2 + B2B2) *n* = 126
Age [years]	45 ±13	46 ±13	46 ±14	46 ±14
BMI [kg/m^2]	26 ±5	26 ±5	28 ±4	27 ±4
SBP [mm Hg]	121 ±15	121 ±15	128 ±12	130 ±13
DBP [mm Hg]	76 ±10	78 ±10	81 ±10	83 ±10
Triglycerides [mg/dl]	109 ±53	103 ±49	148 ±75	134 ±70
Total cholesterol [mg/dl]	193 ±35	199 ±35	193 ±36	196 ±36
HDL cholesterol [mg/dl]	57 ±13	62 ±13*	45 ±9	49 ±11*
LDL cholesterol [mg/dl]	114 ±32	116 ±33	118 ±34	120 ±32
Apolipoprotein AI [mg/dl]	153 ±36	160 ±32	135 ±29	143 ±28
Glucose [mg/dl]	84 ±13	85 ±12	92 ±12	90 ±12
HDL-C/Apo AI	0.374 ±0.075	0.396 ±0.083*	0.351 ±0.078	0.364 ±0.081
TG/HDL-C	2.102 ±1.438	1.797 ±1.355*	3.522 ±2.349	2.861 ±1.868*
AIP	–0.117 ±0.276	–0.171 ±0.22	0.082 ±0.284	–0.015 ±0.228*

*Data are presented as mean ± SD. SBP, DBP – systolic and diastolic blood pressure. AIP (Atherogenic Index of Plasma) was calculated according to the following formula: AIP = log (TG mmol)/ (HDL mmol). Ratio TG/HDL-C calculated in mg/ml. *p < 0.05; differences between B2 allele carriers and B1B1 homozygotes; ANOVA or Kruskal-Wallis test was used.*

was found in women ($p < 0.00001$) and in men ($p = 0.0368$) (Table V). No association of apo AI-75G/A variants with HDL cholesterol percentile ranges was observed (data not shown).

Obesity is known to disturb lipid metabolism and, as expected, lower HDL cholesterol concentrations were found in studied obese subjects than in non-obese subjects. Statistically significant differences in HDL cholesterol were observed between obese and non-obese men ($p = 0.02$; 44 ±9 mg/dl vs. 50 ±11 mg/dl, respectively) and women ($p = 0.001$; 54 ±13 mg/dl vs. 63 ±13 mg/dl, respectively). However, obese and non-obese B2 allele carriers of both sexes had higher HDL cholesterol concentrations than carriers of the B1B1 genotype ($p = 0.161$ for obese men and $p = 0.016$ for obese women; $p = 0.041$ for non-obese men and $p = 0.033$ for non-obese women). Separate analyses performed for obese and non-obese men and women revealed that non-obese women with the A allele (75G/A polymorphism in the apo AI gene) had significantly ($p = 0.027$) higher HDL cholesterol levels than non-obese GG homozygotes, while no differences in obese women ($p = 0.130$) and in men (either obese ($p = 0.142$) or non-obese ($p = 0.261$)) were found.

Crude logistic regression analysis (Table VI) showed that B1B1 homozygotes had 2.36 times (95% CI: 1.62–3.45) higher odds of having low HDL cholesterol (< 25th percentile) than B2 allele carriers, and female B1B1 homozygotes had 3.18 times higher odds (95% CI: 1.99–5.05) while male B1B1 homozygotes had 1.32 times higher odds of having low HDL cholesterol (95% CI: 0.68–2.59). Hypertriglyceridemia (serum TG > 200 mg/dl) significantly increased the risk of low HDL cholesterol (OR = 4.42, 95% CI: 2.51–7.77). Mean serum triglyceride concentrations were higher in men than in women (141 ±72 mg/dl vs. 104 ±50 mg/dl, $p < 0.05$), but occurrence of hypertriglyceridemia (serum TG > 200 mg/dl) was associated with more than 3 times higher chance of having low HDL cholesterol in women (OR = 11.42, 95% CI: 4.06–32.17) than in men (OR = 3.06, 95% CI: 1.38–6.65). As expected, obesity significantly enhances the risk of low HDL cholesterol: in the whole sample (OR = 2.61, 95% CI: 1.75–3.86) and in men (OR = 2.03, 95% CI: 1.01–4.09) and women (OR = 3.00, 95% CI: 1.87–4.83). Low apo AI concentration (apo AI < 25th percentile) also significantly increased the risk – in the whole sample OR = 4.40 (95% CI: 2.56–7.54), in women OR = 4.29 (95% CI: 2.26–8.14) and in men OR = 4.94 (95% CI: 1.76–13.86) – while no statistically significant influence of the 75G/A polymorphism in the apo AI gene was recognized. Variables that were significantly associated with low HDL cholesterol in univariable models were then used in multivariable logistic regression analysis. Multivariable logistic regression analysis revealed that all these variables influenced the risk of having low HDL cholesterol (< 25th percentile), and a significant impact ($p = 0.000$) of B1B1 homozygosity after adjustment for apo AI, BMI and hypertriglyceridemia was noted in women (Table VI). Additionally, in women the impact of high serum triglycerides on HDL cholesterol concentration was almost four times higher than in men (OR = 9.41 vs. OR = 2.64).

Crude estimates indicated that the B2 allele, high apo AI concentration (> 75th percentile), BMI

Table V. Association between high HDL cholesterol (> 75th percentile) and low HDL cholesterol (< 25th percentile) and TaqI CETP genetic variants in women (**A**) and men (**B**)

A

Women	**HDL-C > 75th percentile (> 70 mg/dl) *N* = 81**	**25th percentile ≤ HDL-C ≤ 75th percentile (51–70 mg/dl) *N* = 231**	**HDL-C < 25th percentile (< 51 mg/dl) *N* = 102**
B1B1 *N* = 139	20 (25%)	64 (28%)	55 (54%)
B2 allele carriers (B1B2 + B2B2) *N* = 275	61 (75%)	167 (72%)	47 (46%)

Pearson's chi-square (χ^2) analysis was used, $\chi^2 = 25.368$, df = 2, $p < 0.00001$.

B

Men	**HDL-C > 75th percentile (> 52 mg/dl) *N* = 62**	**25th percentile ≤ HDL-C ≤ 75th percentile (40–52 mg/dl) *N* = 100**	**HDL-C < 25th percentile (< 40 mg/dl) *N* = 45**
B1B1 *N* = 81	16 (26%)	45 (45%)	20 (45%)
B2 allele carriers (B1B2 + B2B2) *N* = 126	46 (74%)	55 (55%)	25 (55%)

Pearson's χ^2 analysis was used, $\chi^2 = 6.601$, df = 2, $p = 0.03686$.

Table VI. Crude estimates and adjusted OR for low HDL cholesterol concentration (< 25th percentile) among women and men

Group	Unadjusted		Adjusted	
	HDL-C < 25th percentile		HDL-C < 25th percentile	
	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value
All subjects:				
CETP B1B1 genotype	2.36 (1.62–3.45)	< 0.0001	2.14 (1.45–3.18)	< 0.0001
Obesity, BMI ≥ 30 kg/m^2	2.61 (1.77–3.86)	< 0.0001	2.24 (1.48–3.37)	< 0.0001
TG > 200 mg/dl	4.42 (2.51–7.77)	< 0.0001	3.39 (1.88–6.12)	< 0.0001
Apo AI < 25th percentile	4.40 (2.56–7.54)	< 0.0001	4.14 (2.32–7.40)	< 0.0001
Women:				
CETP B1B1 genotype	3.18 (2.0–5.05)	< 0.0001	3.29 (2.00–5.39)	< 0.0001
Obesity, BMI ≥ 30 kg/m^2	3.00 (1.9–4.83)	< 0.0001	2.87 (1.72–4.80)	< 0.0001
TG > 200 mg/dl	11.42 (4.06–32.17)	< 0.0001	9.41 (3.19–27.74)	< 0.0001
Apo AI < 25th percentile	4.29 (2.26–8.14)	< 0.0001	5.12 (2.47–10.59)	< 0.0001
Men:				
CETP B1B1 genotype	1.32 (0.68–2.59)	0.4098	1.09 (0.54–2.20)	0.8167
Obesity, BMI ≥ 30 kg/m^2	2.03 (1.01–4.09)	0.005	1.60 (0.76–3.35)	0.209
TG > 200 mg/dl	3.06 (1.38–6.65)	0.005	2.64 (1.17–5.97)	0.0187
Apo AI < 25th percentile	4.94 (1.76–13.8)	0.0021	4.36 (1.48–12.83)	0.0069

For women the cut-off value of HDL cholesterol concentration < 25th percentile was 51 mg/dl. For men the cut-off value of HDL cholesterol concentration < 25th percentile was 40 mg/dl. For women the cut-off value of apo AI concentration < 25th percentile was 136 mg/dl. For men the cut-off value of apo AI concentration < 25th percentile was 122 mg/dl.

< 30 kg/m^2 and serum TG concentration lower than 200 mg/dl significantly increased the chance of high HDL cholesterol (> 75th percentile), and no significant impact of the 75G/A polymorphism in the apo AI gene was confirmed. However, in multivariable logistic regression analysis only high apo AI range and BMI < 30 kg/m^2 were found to significantly enhance the chance of having high HDL cholesterol. In the model adjusted for BMI, apo AI and triglycerides, occurrence of the B2 allele did not have a significant impact (p = 0.076) on high HDL cholesterol (Table VII).

Discussion

Cardiovascular disease is the leading cause of death, and a high level of LDL cholesterol is a major risk factor. However, despite adequate control of LDL cholesterol still a substantial number of patients suffer from cardiovascular events [27, 28]. Therefore, alternative targets to reduce residual cardiovascular risk are of great importance [29]. Low HDL cholesterol is well accepted as one of the major risk factors for cardiovascular events [30, 31]. Observational studies have also shown that each 1 mg/dl rise in HDL cholesterol is associated with a 2–3% drop in coronary artery disease risk [32]. Although increased HDL cholesterol concentration caused by CETP deficiency was not found to be a good risk predictor [15, 17, 33], several CETP genetic variants characterized by high HDL cholesterol were found to be associated with longevity [34]. Involvement of HDL in lipid transport and reverse cholesterol transport and multiple mechanisms of HDL activity outside lipid metabolism make it difficult to relate HDL cholesterol to outcomes affected by HDL functions. Beside levels of HDL cholesterol, other HDL-related biomarkers and the quality of HDL particles have been discussed as more relevant to estimate risk of cardiovascular disease [8, 35]. Also pharmacological therapies for modulating HDL metabolism and functionality have been reviewed recently [4]. In clinical practice HDL cholesterol measurement is commonly used to assess HDL, and nowadays there is no other HDL-related parameter recommended to general practitioners and other physicians. Although different HDL-related measures have been proposed as apo AI concentration or HDL particle measures by NMR, they are still not recommended and are used in specialized centers [36–38]. Recently, there was developed a clinical NMR instrument that allows HDL particle mea-

Table VII. Crude estimates and adjusted OR for high HDL-cholesterol concentration (> 75th percentile) among women and men

Group	Unadjusted		Adjusted	
	HDL-C > 75th percentile		HDL-C > 75th percentile	
	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value
All subjects:				
CETP B2 allele presence	1.86 (1.22–2.83)	0.0038	1.69 (0.95–3.02)	0.076
BMI < 30 kg/m^2	2.87 (1.72–4.79)	0.0001	2.82 (1.29–6.20)	0.0097
TG ≤ 200 mg/dl	5.82 (1.79–18.96)	0.0034	2.87 (0.61–13.54)	0.1801
Apo AI > 75th percentile	6.33 (3.75–10.70)	< 0.0001	6.31 (3.67–10.84)	< 0.0001
Women:				
CETP B2 allele presence	1.70 (0.97–2.95)	0.0609	1.75 (0.81–3.79)	0.1509
BMI < 30 kg/m^2	2.02 (1.08–3.78)	0.0262	2.18 (0.82–5.78)	0.115
TG ≤ 200 mg/dl	5.11 (0.67–38.91)	0.1140	1.44 (0.15–13.42)	0.749
Apo AI > 75th percentile	6.24 (3.16–12.32)	< 0.0001	6.15 (3.08–12.27)	< 0.0001
Men:				
CETP B2 allele presence	2.34 (1.21–4.52)	0.0113	1.59 (0.60–4.18)	0.3416
BMI < 30 kg/m^2	5.38 (2.16–13.39)	0.0003	4.34 (1.02–18.47)	0.0446
TG ≤ 200 mg/dl	8.84 (2.03–38.45)	0.0035	8.79 (0.91–84.82)	0.0576
Apo AI > 75th percentile	8.62 (3.33–22.31)	< 0.0001	10.48 (3.51–31.27)	< 0.0001

For women the cut-off value of HDL cholesterol concentration > 75th percentile was 70 mg/dl. For men the cut-off value of HDL cholesterol concentration > 75th percentile was 52 mg/dl. For women the cut-off value of apo AI concentration > 75th percentile was 177 mg/dl. For men the cut-off value of apo AI concentration > 75th percentile was 153 mg/dl.

surements to be performed routinely in the clinical laboratory [39]. However, evaluation of the clinical usefulness of this assay is needed. Therefore, better understanding of the influence of genetic and metabolic or environmental factors and their interactions on HDL cholesterol could be helpful to more properly relate HDL cholesterol concentrations to clinical outcomes.

In the present study a significant influence of TaqIB polymorphism in the CETP gene on HDL cholesterol concentrations in subjects from the Polish population was documented. According to our knowledge, so far no reliable data on frequency of CETP-TaqI and apo AI 75G/A genetic polymorphisms, and effects of these variants on HDL cholesterol as well as their interaction with other factors influencing HDL level in Polish adults, are available. Our previous preliminary study on the relationships between polymorphisms in CETP and apo AI genes and parameters of lipid metabolism were performed in a relatively small group of subjects [40, 41]. Data of the present study are in agreement with previous reports indicating that the B2 allele is related to decreased CETP mass and activity, and increased HDL cholesterol concentrations and B1B1 carriers show the lowest and B2B2 carriers the highest HDL cholesterol concentrations [42, 43]. B2B2 genotype has been reported to be a protective factor against development of myocardial infarction, and B1B1 homozygotes are likely to have higher risk of CVD [44]. Our data indicate that carriers of the B2 allele of the CETP TaqI polymorphism had 1.86 times higher chance of having high HDL cholesterol (> 75th percentile) than B1B1 homozygotes, while B1B1 homozygotes had 2.36 times higher risk of having low HDL cholesterol (< 25th percentile). However, subjects with low HDL cholesterol as compared to subjects with high HDL cholesterol are often characterized by high serum triglycerides and occurrence of small dense LDL particles as presented based on a very large database of lipids in US adults and children [45]. It underlines the important effect of disturbances in triglyceride-rich lipoprotein metabolism on HDL cholesterol [24]. In the present study obesity and hypertriglyceridemia (triglycerides > 200 mg/dl) significantly modified the effect of CETP TaqI polymorphism on HDL cholesterol. Obese subjects had lower HDL cholesterol levels than non-obese ones, but in obese carriers of the B2 allele HDL cholesterol concentrations were higher than in obese B1B1 homozygotes. Previously, it was suggested that the B2B2 genotype may protect against the HDL

cholesterol lowering associated with obesity [46]. However, it can be hypothesized that even if in obese B2 allele carriers proper HDL cholesterol levels are observed, it may not be related to lower CVD risk. Obesity is commonly accompanied by raised serum triglycerides indicating raised levels of triglyceride rich-lipoproteins which can increase CVD risk [47, 48]. In addition, an increased level of triglyceride-rich particles may be associated with an increased level of apo CIII, which can move to HDL and make HDL particles dysfunctional [29, 49, 50]. Decreased CETP activity associated with the B2 genetic variant reduces removal of cholesterol and remodeling of HDL particles, which can result in accumulation of larger dysfunctional particles. Large HDL particles were found to be associated with increased CVD risk [51, 52].

Although obesity and hypertriglyceridemia significantly influence HDL cholesterol concentrations [50], our data show that in obese and hypertriglyceridemic subjects a significant effect of B1B1 homozygosity on having low HDL cholesterol is observed, as revealed by a multivariable logistic regression analysis. As obesity has become a worldwide epidemic and may reduce or even reverse the observed decline in myocardial infarction incidence and coronary heart disease mortality, the observed interaction between B1B1 homozygosity and obesity may significantly increase CVD risk and may have a significant impact on public health because of the high frequency of this genotype [53]. In addition, the present study indicates that the impact of the B2 allele of the CETP-TaqI polymorphism on having high HDL cholesterol does not seem to be significant when levels of apo AI and triglycerides and BMI are taken into consideration. A significant association between CETP genetic variants causing high HDL cholesterol concentrations and reduced cardiovascular disease risk was reported [54]. However, carriers of the HDL-increasing CETP alleles were characterized by significantly lower LDL cholesterol and serum triglyceride concentrations. Therefore, the impact of genetic, metabolic and environmental factors affecting triglyceride-rich lipoprotein metabolism on HDL cholesterol levels appeared to be more important than the impact of genetic factors causing increased HDL cholesterol levels [55].

There are fewer available data concerning the apo AI MspI (75G/A) polymorphism. The A allele was found to be related to an increase of Apo AI gene expression and serum apo AI concentrations [56]. It was reported that the A allele was a major contributor to inter-individual variability in plasma cholesterol efflux capacity in women [57], and subjects carrying this allele had increased circulating levels of small HDL particles [58]. In the present study no significant influence of 75G/A variants in the apo AI gene on HDL cholesterol was found. Statistical analyses revealed that only in non-obese women was occurrence of the A allele associated with significantly increased HDL cholesterol levels, but the effect of this allele did not persist when in statistical analysis other confounders (such as CETP genetic variants, obesity and hypertriglyceridemia) were taken into account. It seems that the potential influence of 75G/A variants is significantly masked by different genetic, metabolic and environmental factors.

In conclusion, the present study indicates a significant impact of the TaqIB polymorphism in the CETP gene on HDL cholesterol levels in the Polish population, while the effect of the 75G/A polymorphism in the apo AI gene appears not to be significant. Obesity and hypertriglyceridemia significantly interact with CETP TaqI variants by increasing the chance of having low HDL cholesterol.

Acknowledgments

This work was supported by the National Science Centre (grant numbers: NN404087940, 2011/01/N/NZ7/04559) and was carried out using CePT infrastructure financed by the European Union – the European Regional Development Fund within the Operational Program (Innovative Economy for 2007-2013).

Conflict of interest

The authors declare no conflict of interest.

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