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Association between the TaqIB polymorphism in the cholesteryl ester transfer protein gene locus and postprandial plasma lipoprotein levels in heterozygotes for familial hypercholesterolemia

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Abstract

Background: We examined the influence of cholesteryl ester transfer protein TaqIB polymorphism on triglyceride (TG) response to an oral fat tolerance test (OFTT) in patients heterozygous for familial hypercholesterolemia (hFH).
Methods: We genotyped 67 hFH patients (32 men and 35 postmenopausal women) who were subjected to an OFTT.
Results: All B1 allele carriers had lower high-density lipoprotein cholesterol (HDL-C) levels ($p=0.013$) and higher postprandial TG response at 6 and 8 h ($p=0.05$ and $p=0.04$, respectively) compared to B2 allele carriers. Multiple regression analysis showed that in the hFH group with a positive response, the presence of the B2 allele was significantly related to lower levels of TG-area under the curve (AUC) ($p<0.01$) compared to B1, adjusting for age, gender and body mass index. In the hFH group with a negative response, although age and female gender had a significant effect on TG-AUC levels ($p<0.01$ for both), the allele type was not significantly related to the TG-AUC levels ($p=0.99$).
Conclusions: B2 carriers had a lower postprandial TG response compared to B1 carriers. There were no differences in TG levels between B1 and B2 carriers in patients with a negative OFTT response. Therefore, at higher TG concentration, the B2 allele may protect against an exaggerated postprandial TG increase and subsequent lowering of HDL-C.
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Introduction

Many studies have shown that hypertriglyceridemia plays a role in the development of premature atherosclerosis (1–3). Disturbances in triglyceride (TG) metabolism are characterized by postprandial accumulation of TG-rich lipoprotein remnants and have been reported in various populations with elevated vascular risk (4–10). Familial hypercholesterolemia (FH) is an autosomal codominant single-gene disorder caused by mutations in the low-density lipoprotein (LDL) receptor gene. The clinical manifestations vary among patients. However, patients heterozygous for FH (hFH) are characterized by an increase in plasma LDL-cholesterol (LDL-C) concentrations, tendon xanthomata and premature coronary heart disease (CHD) (11). FH in combination with disturbances in postprandial lipoprotein metabolism is associated with a higher risk of CHD (12). Animal studies have shown that LDL receptor deficiency is associated with a delayed clearance of chylomicron remnants since, among other receptors, the LDL receptor is used for remnant hepatic uptake (13–15). We (16, 17) and others (7, 12, 18) have demonstrated a significant postprandial increase in TG-rich lipoproteins in hFH patients compared to normolipidemic controls.

Cholesteryl ester transfer protein (CETP) plays a major role in the remodeling of lipoprotein particles by mediating the transfer of cholesteryl ester from high-density lipoprotein (HDL) to apolipoprotein B-containing lipoproteins in exchange for TG. When the level of TG-rich lipoproteins is normal, CETP transfers of HDL cholesteryl esters are directed with preference towards LDL particles (19). In contrast, when the level of TG-rich lipoproteins is increased, CETP transfers of HDL cholesteryl esters are directed towards larger very low-density lipoprotein particles, and there are high net transfer rates of TG to LDL and HDL (20). TG-enriched LDL and HDL are substrates for hepatic lipase, giving rise to small dense LDL and HDL particles, respectively (20). Small dense LDL particles have proatherogenic properties (e.g., increased arterial wall retention and susceptibility to oxidation) (19, 21), while small dense HDL particles are more prone to catabolism. TaqIB polymorphism has been found to account for 5.8% of the variance in HDL cholesterol (HDL-C) (22). Subjects with the B2 allele usually have

lower levels of CETP, higher levels of HDL-C and reduced risk of CHD in males compared to B1 subjects (23).

To date, only a few studies have evaluated the postprandial TG response of hFH patients (7, 16–18). The objective of the present study was to assess the TG response to oral fat loading and its relationship to CETP TaqIB polymorphism in hFH patients.

Materials and methods

Subjects

We genotyped 67 hFH patients (32 men and 35 postmenopausal women) who were previously subjected to an oral fat tolerance test (OFTT). All patients were from the Lipid Clinic of the Onassis Cardiac Surgery Center, Athens, Greece. All patients gave informed consent and the center's Institutional Review Board approved the study. The diagnosis for hFH was based on the following clinical criteria: a) total cholesterol >290 mg/dL (7.5 mmol/L) and LDL-C >190 mg/dL (4.9 mmol/L); b) presence of tendon xanthomata in the patient or in a first- or second-degree relative; and c) history of premature vascular disease in a first-degree relative >60 years or in a second-degree relative >50 years (11). None of the patients were on hypolipidemic drug treatment prior to participating in the study, since it was their first visit to the hypolipidemic clinic. In addition, none of the patients had previously undergone any diagnostic test to exclude CHD. Therefore, at the time of the OFTT none of the patients were clinically diagnosed with CHD and were not on any relevant medication. However, after the OFTT all patients were further evaluated for possible CHD by performing a stress test. Two of the patients were found to have a positive stress test and were diagnosed with CHD by angiography. Some of these results have been published elsewhere (24). Smokers were defined as current or ex-smokers. Non-smokers were defined as those who never smoked. Women were defined as postmenopausal when they reported their last menses to have been at least 12 months earlier. None were on hormone replacement therapy. Diabetes mellitus and arterial hypertension were not present in any of the patients. Moreover, none of the patients fulfilled the criteria for the metabolic syndrome according to the National Cholesterol Education Programme-Adult Treatment Panel III criteria (25). Heavy drinking, liver and renal disease, obesity and diabetes mellitus, hypertension, hypothyroidism and professional sports activity were exclusion criteria.

The patients were divided into two groups: 1) the hFH group with a positive OFTT response (hFH-P) consisted of 36 patients (17 men and 19 women); and 2) the hFH group with a negative OFTT response (hFH-N) consisted of 31 patients (15 men and 16 women). Allelic effects on the clinical and metabolic measurements were examined by grouping patients according to their genotype and allele carrier status. The B1B2 was grouped with B1B1 genotype to examine the absolute effect of the B2 variant on OFTT, excluding any possible B1 influence.

TG response to a fatty meal was considered positive when any of the postprandial TG concentrations (at 2, 4, 6 or 8 h) were higher than the highest TG concentration (220 mg/dL or 2.5 mmol/L) observed in healthy subjects in our previous studies (9, 16).

Determination of blood lipids and glucose

Plasma total cholesterol, TG and HDL-C were measured using enzymatic colorimetric methods on a Roche Integra Biochemical analyzer with commercially available kits (Roche Diagnostics GmbH, Mannheim, Germany). Serum LDL-C levels were calculated using the Friedewald formula (26) in patients with TG levels <400 mg/dL (4.5 mmol/L). Apolipoprotein A and B and lipoprotein(a) were measured by nephelometry (Nephelometer BN-100, Behring, Marburg, Germany). Blood glucose was measured by the hexokinase method with a Dade Behring reagent on a Dimension instrument (Dade Behring, Liederbach, Germany). Blood insulin was measured using an IMX Abbott Diagnostics instrument (Wiesbaden, Germany). All samples were analyzed within 24 h. The OFTT protocol has been described elsewhere (9).

Body mass index (BMI) was calculated as weight divided by height squared and expressed in kg/m^2. We assessed whole-body insulin resistance using the following formulas: the homeostasis model assessment for insulin resistance (HOMA-IR) = fasting glucose $\times$ fasting insulin/22.5, and the quantitative insulin-sensitivity check index (QUICKI) parameter as 1/log insulin + log glucose in mg/dL.

Genotyping

Genotyping of CETP TaqIB polymorphism was performed by PCR and restriction fragment length polymorphism analysis (27). Briefly, each PCR reaction was performed using 500 ng of genomic DNA in 25 μL containing 50 mM KCl, 10 mM Tris HCl (pH 8.8), 200 μM dNTPs, 1.0–1.5 mM $MgCl_2$, 12.5–25 pmol of each primer and 0.75 U of Taq polymerase (Keymed Srl., Rome, Italy). The intron 1 region containing the TaqIB polymorphism was amplified using the forward oligo, 5′-CAC TAG CCC AGA GAG GGA GTG CC-3′; and the reverse oligo, 5′-CTG AGC CCA GCC GCA CAC TAA C-3′, giving a fragment of 535 bp in length (27). The PCR conditions were an initial denaturation at 95°C for 6 min, followed by 30 cycles at 95°C for 30 s, 65°C for 30 s, and 72°C for 30 s and finally at 72°C for 5 min. For the detection of TaqIB polymorphism, 5 μL of the PCR product was digested with 5 U of TaqI (New England Biolabs, Frankfurt, Germany) at 65°C overnight, giving 174- and 361-bp fragments in the presence of the TaqI site.

Statistical analysis

Power analysis showed that the number of enrolled participants needed to evaluate two-sided differences $>20\%$ in the investigated parameters between the study groups, achieving statistical power of 80% at a probability level of <0.05, was 31 individuals in each group. Values of numerical characteristics were tested for normality using the Shapiro-Wilk test. All variables deviated from normality; therefore, nonparametric statistical methods were used. The Mann-Whitney U-test was used for comparison of numerical values between two groups, while the Kruskal-Wallis H-test was used for comparison of numerical values between three groups. The comparison of clinical categorical variables was performed using the Pearson χ^2 statistic. Areas under the curve (AUC) for serial measurements of TG levels at baseline and after the OFTT were calculated using the trapezoid rule. To assess the influence of alleles on TG levels, we performed multiple median (least absolute value) regression analysis adjusting for age, gender and BMI, where the AUC-TG was the dependent variable and the aforementioned variables the independent (explanatory) variables, since they did not distribute normally. To assess any interaction between postprandial response and allele carrier status, an interaction term between the aforementioned variables was constructed. This term was included in the multiple regression model prior to further analysis to assess the significance of this

interaction, and therefore the tenability of splitting our group according to postprandial response. The t-statistic was calculated to assess the significance of each dependent variable. Statistical significance was set at $p<0.05$. Data were analyzed using STATA™ (Version 9.0, Stata Corporation, College Station, TX, USA).

Results

All participants ingested the individually calculated OFTT and tolerated it well. OFTT of FH patients with a positive and a negative response are shown in Figure 1.

Baseline characteristics

The clinical characteristics of the hFH-P and hFH-N patients are shown in Table 1. Of the 67 hFH patients, 54% had an abnormal OFTT response. The hFH-P group had significantly lower HDL-C levels than the hFH-N group. Additionally, the hFH-P group had significantly higher BMI and waist circumference than the hFH-N group. HOMA-IR values were significantly higher in the hFH-P group than in the hFH-N group. Of the hFH-P and hFH-N groups, 58% and 55% were smokers, respectively.

Postprandial TG concentrations in the two groups

The hFH-P and hFH-N groups showed significant differences in fasting and postprandial TG levels at all time points, as expected from the study design (Table 1). Accordingly, the TG-AUC and incremental AUC (i-AUC) values were higher in the hFH-P group compared to the hFH-N group.

Genotypes and allele frequencies in the studied groups

The frequency of genotypes of the whole population was 26.9% for B1B1, 55.2% for B1B2 and 17.9% for B2B2, and was in Hardy-Weinberg equilibrium. The frequency of allele carriers of the whole population was 54.5% for B1 and 45.5% for B2. There was no difference in genotype (B1B1, B1B2 and B2B2) and allele carrier (B1 and B2) frequency distribution between the hFH-P and hFH-N groups (Table 1).

Fasting and postprandial lipid and lipoprotein profile according to allele carriers and genotypes

Whole study cohort B1 allele carriers showed a trend towards higher fasting TG levels compared to B2 allele carriers, although this difference was not significant ($p=0.06$). Additionally, they had significantly higher TG levels at 6 and 8 h after fat loading compared to B2 allele carriers ($p=0.05$ and $p=0.04$, respectively). B2 allele carriers had higher HDL-C levels compared to B1 allele carriers ($p=0.01$). On splitting the population into the three genotypes, a significant difference in HDL levels was observed (Table 2). Specifically, the patients with B1B1 genotype had significantly lower HDL-C levels compared to both the B1B2 genotype ($p=0.003$) and B2B2 genotype ($p=0.013$). Additionally, significant differences in total cholesterol, LDL and apolipoprotein B were observed (Table 2).

After the three-genotype group analysis, B1B2 was grouped with B1B1 to examine the absolute effect of the B2 variant on OFTT, excluding any possible B1 influence. Comparison of B2B2 with B1B1/B1B2 genotypes revealed no significant difference either in postprandial lipemia or in HDL levels

hFH-P group B1 allele carriers had higher fasting TG levels compared to B2 allele carriers ($p=0.04$). B1 allele carriers had higher TG levels at 2 and 6 h after fat loading ($p=0.05$ and $p=0.02$, respectively) compared to B2 allele carriers. The B1 allele carriers had higher TG-AUC levels than B2 allele carriers ($p=0.03$).

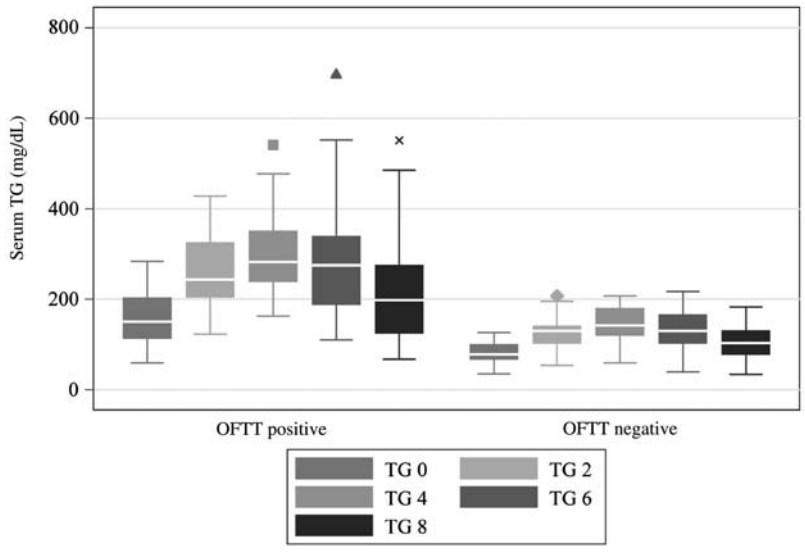

Figure 1 OFTT of FH patients with a positive and a negative response.
TG 0, triglyceride levels before the meal; TG 2, 4, 6, 8, triglyceride levels at 2, 4, 6, 8 h after the fat load, respectively. To convert TG from mg/dL to mmol/L, multiply by 0.0113.

Table 1 Clinical and metabolic characteristics of the study cohort.

Characteristics	hFH-P (n=36)	hFH-N (n=31)	p-Value
Age, years	50 (24)	43 (23)	0.89
BMI, kg/m^2	25.5 (4.2)	23.6 (3.0)	0.03
Waist, cm	89 (18)	81 (17)	0.04
TC, mg/dL	313 (43)	319 (72)	0.84
TG0, mg/dL	151 (92)	78 (32)	<0.001
TG2, mg/dL	244 (122)	130 (40)	<0.001
TG4, mg/dL	282 (111)	142 (62)	<0.001
TG6, mg/dL	275 (150)	130 (62)	<0.001
TG8, mg/dL	198 (163)	103 (52)	<0.001
TG-AUC, mg/dL/h	1958 (879)	991 (296)	<0.001
TG i-AUC, mg/dL/h	795 (445)	363 (145)	<0.001
HDL, mg/dL	42 (20)	54 (24)	0.001
LDL, mg/dL	232 (47)	242 (62)	0.58
Apolipoprotein A, mg/dL	140 (39)	156 (45)	0.13
Apolipoprotein B, mg/dL	164 (35)	154 (40)	0.21
Lipoprotein(a), mg/dL	18 (18)	15 (21)	0.91
Glucose 0, mg/dL	91 (16)	86 (14)	0.09
Glucose 2, mg/dL	88 (17)	88 (14)	0.80
Insulin 0, μU/mL	6.3 (7.3)	4.1 (2.9)	0.06
Insulin 2, μU/mL	11.9 (6.6)	7.7 (12.7)	0.13
HOMA-IR	1.4 (1.8)	0.9 (0.6)	0.04
QUICKI	3.2 (0.7)	3.6 (0.7)	0.11
TaqIB genotype frequency, %			
B1B1	33.3	19.4	0.33
B1B2	47.2	64.5	
B2B2	19.4	16.1	
TaqIB allele frequency, %			
B1	56.9	51.6	0.54
B2	43.1	48.4	

Data are presented as median (interquartile range). hFH-P, heterozygous familial hypercholesterolemia (hFH) group with positive oral fat tolerance test (OFTT) response; hFH-N, hFH group with negative OFTT response; BMI, body mass index; TC, total cholesterol; TG, triglyceride; TG-AUC, triglyceride-area under the curve; TG i-AUC, TG incremental AUC; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol; HOMA-IR, homeostasis model assessment for insulin resistance; QUICKI, quantitative insulin-sensitivity check index. To convert TC, HDL and LDL from mg/dL to mmol/L, multiply by 0.0259; TG from mg/dL to mmol/L, multiply by 0.0113; glucose from mg/dL to mmol/L, multiply by 0.0555; lipoprotein(a) from mg/dL to μmol/L, multiply by 0.0357; apolipoproteins A and B from mg/dL to g/L, multiply by 0.01; and insulin from μU/mL to pmol/L, multiply by 6.945.

Patients with the B1B1 or B1B2 genotype had higher TG levels at 4 h, as well as higher TG-AUC levels compared to patients with the B2B2 genotype (Table 3). Additionally, B1B1/B1B2 patients showed a trend towards higher TG levels at 6 h compared to B2B2 patients. Baseline glucose levels were significantly higher in B1B1/B1B2 patients. There was a trend towards higher HOMA-IR values in B1B1/B1B2 patients.

hFH-N group No differences were found between allele carriers. B1B1/B1B2 patients had higher TG levels at 6 h and lower baseline glucose levels compared to B2B2 patients (Table 4).

Multiple regression analysis (Table 5)

In the hFH-P group, the presence of the B2 allele was significantly related to lower TG-AUC levels ($p<0.01$) compared to B1, adjusting for age (positive relationship, $p<0.01$), gender (females had significantly lower levels of TG-AUC, $p<0.01$) and BMI (no significant effect, $p=0.99$).

In the hFH-N group, although age and female gender had a significant effect on TG-AUC levels (coefficients of 11.52 and -983.50, $p<0.01$ for both), allele type was not significantly related to TG-AUC levels in this group ($p=0.99$). BMI did not predict our dependent variable ($p=0.95$).

In the overall sample, the negative effect of the B2 allele on TG-AUC remained significant, even after adjusting for gender, age, BMI and subgroup (hFH-P and hFH-N). The aforementioned parameters were also significant predictors of TG-AUC, while the interaction term between postprandial response and allele carrier status had a significant effect on TG-AUC in the total sample, justifying our choice to conduct multiple comparisons between the postprandial response groups and genotype stratification.

Discussion

We analyzed the association between CETP TaqIB genotype and fasting and postprandial lipid levels in hFH patients. B1 allele carriers with a positive response to fat loading had higher fasting and postprandial TG compared to B2 allele carriers. Furthermore, all B1 allele carriers compared to B2 allele carriers showed a trend towards higher fasting TG

Table 2 Clinical and metabolic characteristics of the whole group of FH patients based on genotype.

Characteristics	B1B1 (n=18)	B1B2 (n=37)	B2B2 (n=12)	p-Value
Age, years	49 (29)	50 (25)	38 (22)	0.28
Men	8 (25%)	17 (53.1%)	7 (21.9%)	0.717
Women	10 (28.6%)	20 (57.1%)	5 (14.3%)	0.717
BMI, kg/m^2	25.7 (3.9)	24.3 (4.5)	24.2 (1.9)	0.32
Waist, cm	90 (17)	84 (19)	80 (8)	0.56
TC, mg/dL	304 (68)	329 (64)	304 (32)	0.05
TG0, mg/dL	149 (103)	99 (58)	98 (64)	0.11
TG2, mg/dL	226 (149)	168 (152)	169 (87)	0.53
TG4, mg/dL	262 (187)	201 (136)	209 (147)	0.45
TG6, mg/dL	269 (210)	170 (149)	142 (138)	0.11
TG8, mg/dL	182 (151)	134 (96)	110 (117)	0.10
TG-AUC, mg/dL/h	1908 (1242)	1263 (842)	1399 (836)	0.23
TG i-AUC, mg/dL/h	635 (706)	497 (444)	399 (548)	0.44
HDL, mg/dL	43 (12)	51 (25)	55 (28)	0.01
LDL, mg/dL	231 (49)	255 (49)	224 (31)	0.04
Apolipoprotein A, mg/dL	134 (35)	150 (42)	154 (57)	0.21
Apolipoprotein B, mg/dL	148 (27)	170 (47)	144 (26)	0.01
Lipoprotein(a), mg/dL	15 (62)	16 (21)	19 (19)	0.97
Glucose 0, mg/dL	87 (15)	89 (15)	88 (17)	0.99
Glucose 2, mg/dL	84 (16)	89 (18)	90 (13)	0.27
Insulin 0, μU/mL	8.0 (6.0)	4.9 (5.5)	4.6 (2.5)	0.26
Insulin 2, μU/mL	8.3 (7.1)	11.9 (13.3)	8.5 (6.5)	0.36
HOMA-IR	1.7 (1.1)	1.0 (1.3)	0.9 (0.6)	0.25
QUICKI	3.0 (0.7)	3.4 (0.7)	3.4 (0.5)	0.22

Data are presented as median (interquartile range). FH, familial hypercholesterolemia; BMI, body mass index; TC, total cholesterol; TG, triglyceride; TG-AUC, triglyceride-area under the curve; TG i-AUC, TG incremental AUC; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol; HOMA-IR, homeostasis model assessment for insulin resistance; QUICKI, quantitative insulin-sensitivity check index. To convert TC, HDL and LDL from mg/dL to mmol/L, multiply by 0.0259; TG from mg/dL to mmol/L, multiply by 0.0113; glucose from mg/dL to mmol/L, multiply by 0.0555; lipoprotein(a) from mg/dL to μmol/L, multiply by 0.0357; apolipoproteins A and B from mg/dL to g/L, multiply by 0.01; and insulin from μU/mL to pmol/L, multiply by 6.945.

Table 3 Clinical and metabolic characteristics of the hFH group with positive OFTT response based on genotype.

Characteristics	B1B1/B1B2 (n=29)	B2B2 (n=7)	p-Value
Age, years	50 (26)	40 (22)	0.28
BMI, kg/m^2	25.7 (4.6)	24.2 (2.0)	0.27
Waist, cm	93 (16)	85 (9)	0.13
TC, mg/dL	313 (54)	306 (33)	0.31
TG0, mg/dL	157 (88)	124 (88)	0.12
TG2, mg/dL	269 (121)	213 (44)	0.08
TG4, mg/dL	318 (131)	268 (62)	0.05
TG6, mg/dL	305 (142)	237 (115)	0.06
TG8, mg/dL	207 (155)	160 (102)	0.11
TG-AUC, mg/dL/h	2093 (851)	1623 (451)	0.04
TG i-AUC, mg/dL/h	821 (430)	638 (548)	0.16
HDL, mg/dL	42 (18)	40 (24)	0.47
LDL, mg/dL	232 (44)	228 (45)	0.51
Apolipoprotein A, mg/dL	145 (33)	137 (57)	0.91
Apolipoprotein B, mg/dL	166 (47)	144 (37)	0.05
Lipoprotein(a), mg/dL	15 (13)	23 (39)	0.47
Glucose 0, mg/dL	93 (14)	81 (12)	0.03
Glucose 2, mg/dL	88 (20)	88 (18)	0.71
Insulin 0, μU/mL	8.4 (7.8)	4.6 (3.4)	0.10
Insulin 2, μU/mL	12.4 (7.3)	10.0 (5.4)	0.17
HOMA-IR	1.8 (1.8)	0.9 (0.8)	0.06
QUICKI	3.1 (0.7)	3.4 (0.8)	0.10

Data are presented as median (interquartile range). hFH, heterozygous familial hypercholesterolemia; OFTT, oral fat tolerance test; BMI, body mass index; TC, total cholesterol; TG, triglyceride; TG-AUC, triglyceride-area under the curve; TG i-AUC, TG incremental AUC; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol; HOMA-IR, homeostasis model assessment for insulin resistance; QUICKI, quantitative insulin-sensitivity check index. To convert TC, HDL and LDL from mg/dL to mmol/L, multiply by 0.0259; TG from mg/dL to mmol/L, multiply by 0.0113; glucose from mg/dL to mmol/L, multiply by 0.0555; lipoprotein(a) from mg/dL to μmol/L, multiply by 0.0357; apolipoproteins A and B from mg/dL to g/L, multiply by 0.01; and insulin from μU/mL to pmol/L, multiply by 6.945.

Table 4 Clinical and metabolic characteristics of the hFH group with negative OFTT response based on genotype.

Characteristics	B1B1/B1B2 (n=26)	B2B2 (n=5)	p-Value
Age, years	46 (25)	35 (25)	0.33
BMI, kg/m^2	23.6 (3.3)	24.2 (2.9)	0.76
Waist, cm	82 (20)	80 (8)	0.89
TC, mg/dL	329 (86)	291 (45)	0.16
TG0, mg/dL	81 (25)	74 (52)	0.75
TG2, mg/dL	128 (44)	130 (26)	0.91
TG4, mg/dL	146 (57)	120 (80)	0.21
TG6, mg/dL	139 (55)	102 (54)	0.03
TG8, mg/dL	112 (54)	80 (57)	0.11
TG-AUC, mg/dL/h	1032 (302)	858 (307)	0.13
TG i-AUC, mg/dL/h	376 (125)	308 (214)	0.18
HDL, mg/dL	52 (26)	63 (22)	0.23
LDL, mg/dL	255 (57)	222 (32)	0.06
Apolipoprotein A, mg/dL	153 (42)	174 (53)	0.34
Apolipoprotein B, mg/dL	157 (48)	137 (49)	0.06
Lipoprotein(a), mg/dL	15 (27)	13 (13)	0.43
Glucose 0, mg/dL	84 (14)	97 (12)	0.03
Glucose 2, mg/dL	87 (19)	93 (13)	0.29
Insulin 0, μU/mL	4.4 (4.2)	4 (4.1)	0.90
Insulin 2, μU/mL	8.4 (15.6)	7.1 (12.7)	0.66
HOMA-IR	0.8 (0.7)	0.88 (0.8)	0.71
QUICKI	3.5 (1.0)	3.61 (1.1)	0.71

Data are presented as median (interquartile range). hFH, heterozygous familial hypercholesterolemia; OFTT, oral fat tolerance test; BMI, body mass index; TC, total cholesterol; TG, triglyceride; TG-AUC, triglyceride-area under the curve; TG i-AUC, TG incremental AUC; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol; HOMA-IR, homeostasis model assessment for insulin resistance; QUICKI, quantitative insulin-sensitivity check index. To convert TC, HDL and LDL from mg/dL to mmol/L, multiply by 0.0259; TG from mg/dL to mmol/L, multiply by 0.0113; glucose from mg/dL to mmol/L, multiply by 0.0555; lipoprotein(a) from mg/dL to μmol/L, multiply by 0.0357; apolipoproteins A and B from mg/dL to g/L, multiply by 0.01; and insulin from μU/mL to pmol/L, multiply by 6.945.

levels and significantly higher TG values at 6 and 8 h, independently of their response to fat loading. Also, patients with the B1B2 genotype had significantly higher HDL-C levels compared to the B1B1 genotype.

The trend towards higher fasting TG levels observed in our B1 allele carriers was also reported by others (28) and in the Framingham study (23) (trend towards higher TG in men with B1B1 genotype, p=0.059). However, the subjects involved in the Veterans Affairs HDL Cholesterol Interventional Trial (VA-HIT) did not show any differences in fasting TG levels between genotypes of CETP TaqIB (29). Also, others

Table 5 Median (least absolute value) multiple regression results by groups.

Explanatory variable	Coefficient	Standard error	p-Value	95% Confidence interval	
hFH-P group					
B2 vs. B1	−485	103.35	<0.01	−690.79	−278.21
Age	27	6.70	<0.01	13.13	39.87
Women vs. men	−984	173.76	<0.01	−1330.33	−636.67
BMI	0.01	20.32	0.99	−40.55	40.55
Constant	3008	548.43	<0.01	1913.33	4102.68
hFH-N group					
B2 vs. B1	0.01	72.20	0.99	−144.69	144.69
Age	12	3.16	<0.01	5.18	17.86
Women vs. men	−257	108.44	0.02	−474.07	−39.41
BMI	−1	17.86	0.95	−36.94	34.65
Constant	886	491.03	0.08	−98.50	1869.59
Total sample					
B2 vs. B1	−561.8	46.6	<0.001	−654.0	−469.7
hFH-N group vs. hFH-P group	−1241.2	49.9	<0.001	−1339.9	−1142.4
Postprandial response X carrier allele status interaction term (B2 and hFH-N group vs. the rest)	561.8	69.3	<0.001	424.6	699.1
Age	14.7	2.0	<0.001	10.8	18.6
Women vs. men	−486.5	56.7	<0.001	−598.8	−374.3
BMI	−26.8	7.2	<0.001	−41.0	−12.6
Constant	2972.0	199.1	<0.001	2577.9	3366.0

hFH-P, heterozygous familial hypercholesterolemia (hFH) group with positive oral fat tolerance test (OFTT) response; hFH-N, hFH group with negative OFTT response; BMI, body mass index.

did not find any difference between allele carriers and plasma TG levels (30). One explanation could involve the selection of the VA-HIT study population, which comprised patients with low HDL-C levels and normal total cholesterol, LDL-C and TG levels compared to our hFH patients, for whom selection was made on the basis of fasting LDL-C [> 190 mg/dL (4.9 mmol/L)]. Furthermore, the frequency of allele carriers in the VA-HIT study was different compared to those in our study (B1, 60.4% vs. 54.5% and B2, 39.6% vs. 45.5%, respectively). The frequency of allele carriers in our population was similar to that in the Framingham population (23) (B1 55.6% and B2 44.4%), of the EARS population (31) (B1 55.7% and B2 44.3%) and of patients with FH (B1 57% and B2 43%) (32). Moreover, subjects with the B1B1 genotype in the VA-HIT study (29) showed the greatest reduction in plasma TG levels during gemfibrozil therapy, although baseline TG levels were very similar in the B1B1, B1B2 and B2B2 groups. The trend towards higher fasting TG levels in B1 allele carriers compared to B2 allele carriers, which was found in the whole population, was even more pronounced for B1 allele carriers with hFH-P ($p = 0.04$) compared to hFH-N, which represents the novelty of the present study. Specifically, B1 allele carriers with hFH-P had higher TG levels 2 and 6 h after fat loading ($p = 0.05$ and $p = 0.02$, respectively) compared to B2 allele carriers with hFH-P.

Furthermore, multivariate analysis showed that the presence of the B2 allele was significantly related to lower levels of TG-AUC ($p < 0.01$). On the other hand, no differences in TG-AUC were found between B1 and B2 allele carriers with hFH-N. The explanation for these findings could be that patients with higher TG have higher levels and activity of CETP, which fits with the phenotype of B1 allele carriers. The higher TG levels in B1 allele carriers are consistent with lower HDL-C levels also found in our study. Subjects homozygous for the B1 allele in the Framingham study (23) had a higher level of CETP and lower HDL-C levels when compared to B2 allele carriers (subjects with the B1B2 or B2B2 genotype). In our study, we did not measure CETP mass and activity. However, Noone et al. found that B1 allele carriers had increased mass and activity of CETP at 6 h after fat loading compared to B2 allele carriers (33). This finding is similar to our results (higher TG 6 and 8 h after fat loading in B1 allele carriers compared to B2; $p = 0.05$ and $p = 0.042$, respectively). This was noted in other studies as well. Tall et al. found a 1.1–1.7-fold increase in CETP in response to a 135-g fat meal (34). It has been shown by others (35, 36) and by the current study that carriers of the B1 allele have a more atherogenic lipid profile (low HDL-C and increased TG, exaggerated and delayed clearance of TG after a fat meal) than carriers of the B2 allele, which should lead to increased cardiovascular risk. Furthermore, Hogue et al. reported that a high plasma CETP concentration was associated with higher risk of having small-diameter particles of LDL in hFH patients, suggesting that CETP-induced remodeling of LDL is dependent on the number of TG-rich lipoproteins (37). In agreement, a positive association between carotid intima-media thickness and CETP has been found (38). Also, we previously found that CETP may be associated with the severity of CHD (39). Considerable debate has taken place regarding the atherogenicity of CETP TaqIB. There are studies suggesting that even though B2 allele carriers have higher HDL-C levels than B1 allele carriers, paradoxically they have an increased risk of CHD (40), in agreement with other studies (41, 42). However, a meta-analysis of seven studies reported a lower cardiovascular risk in B2 compared to B1 homozygotes (43).

CETP gene polymorphism may be important, since patients with FH and low CETP levels have an improved lipid profile after statin treatment compared to FH patients with higher CETP levels (44).

In conclusion, the B2 allele was significantly related to lower levels of TG-AUC compared to B1 carriers in the hFH-P group. However, allele type was not significantly related to TG-AUC levels in patients with a negative OFTT. The lowering effect of B2 on CETP mass and activity reported by others may thus become apparent when TG levels are higher than normal. Therefore, at higher TG concentrations, the B2 allele may protect against exaggerated postprandial TG increases and subsequent lowering of HDL-C concentrations.

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