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Original Research

Association of the CETP Taq1B and LIPG Thr111Ile Polymorphisms with Glycated Hemoglobin and Blood Lipids in Newly Diagnosed Hyperlipidemic Patients

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ABSTRACT

Objective: To examine the association of 2 common polymorphisms in high-density lipoprotein (HDL)-related genes, namely, cholesterol ester transfer protein CETP Taq1B (rs708272) and endothelial lipase LIPG Thr111Ile (rs2000813), with glycated hemoglobin (A1C), blood lipid levels and the risk for type 2 diabetes in a group of hyperlipidemic patients from northern Greece.
Methods: We categorized 175 patients with hyperlipidemia into 2 subgroups according to the presence or absence of type 2 diabetes, defined as a recent diagnosis, A1C >6.5% and/or fasting glucose >126 mg/dL. Genotypes for the 2 polymorphisms studied were determined by polymerase chain reaction-restriction fragment length polymorphism. Both polymorphisms were analyzed by multivariate and univariate analyses of baseline A1C levels and plasma lipids. The genotype and allele frequencies of the 2 subgroups were compared.
Results: The CETP Taq1B polymorphism was associated with HDL-cholesterol (HDL-C) and A1C levels, but this association was affected by type 2 diabetes; the association with A1C levels was significant only in type 2 diabetes (p=0.005), whereas the association with HDL-C occurred only in the subgroup without type 2 diabetes (p<0.001). LIPG Thr111Ile did not affect plasma HDL-C or A1C levels independently but appeared to modulate their association with CETP Taq1B, and LIPG 111IleIle homozygotes tended to be present at a higher frequency in the hyperlipidemic patients with type 2 diabetes compared to the hyperlipidemic patients without type 2 diabetes (p=0.056).
Conclusions: In hyperlipidemic patients, apart from its known association with HDL-C, CETP Taq1B is also associated with A1C levels, and both associations are modified by type 2 diabetes and LIPG Thr111Ile.

Mots clés :
protéine de transfert des esters de cholestérol
lipase endothéliale
polymorphismes des gènes, hémoglobine glyquée
lipides plasmatiques
diabète de type 2

RÉSUMÉ

Objectif : Examiner l'association entre 2 polymorphismes communs dans les gènes liés au cholestérol à lipoprotéines de haute densité (HDL), à savoir, le Taq1B de la protéine de transfert des esters de cholestérol, CETP (rs708272) et le Thr111Ile de la lipase endothéliale, LIPG (rs2000813), et les concentrations sanguines de l'hémoglobine glyquée (A1c) et des lipides, et le risque de diabète de type 2 chez un groupe de patients hyperlipidémiques du nord de la Grèce.
Méthodes : Nous avons réparti 175 patients atteints d'hyperlipidémie en 2 sous-groupes selon la présence ou l'absence de diabète de type 2, défini par un diagnostic récent, d'une A1c>6,5 % et/ou d'une glycémie à jeun>126 mg/dl. Nous avons déterminé les génotypes des 2 polymorphismes étudiés par la technique de réaction en chaîne de la polymérase–polymorphisme de longueur des fragments de restriction. Nous avons analysé les 2 polymorphismes au moyen d'analyses multivariées et univariées des concentrations initiales d'A1c et des lipides plasmatiques. Nous avons comparé les fréquences des génotypes et des allèles des 2 sous-groupes.

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Résultats : Le polymorphisme Taq1B de la CETP était associé aux concentrations de cholestérol HDL (HDL-C) et d'A1c, mais cette association était affectée par le diabète de type 2; l'association avec les concentrations d'A1c était seulement significative lors de diabète de type 2 (p=0,005), alors que l'association avec le HDL-C apparaissait seulement chez le sous-groupe atteint du diabète de type 2 (p<0,001). Le Thr111Ile de la LIPG n'affectait pas les concentrations plasmatiques du HDL-C ou de l'A1c de façon indépendante, mais semblait moduler leur association avec le Taq1B de la CEPT, et les homozygotes 111IleIle de la LIPG avaient tendance à être présents selon une fréquence plus élevée chez les patients hyperlipidémiques atteints du diabète de type 2 comparativement aux patients hyperlipidémiques non atteints du diabète de type 2 (p=0,056).
Conclusions : Chez les patients hyperlipidémiques, le Taq1B de la CETP, excepté son association connue avec le HDL-C, est également associé aux concentrations d'A1c, mais les deux associations sont modifiées par le diabète de type 2 et le Thr111Ile de la LIPG.

Introduction

Dyslipidemia, mainly in the form of (but not restricted to) hypertriglyceridemia and low blood high-density lipoprotein cholesterol (HDL-C), is a feature of the metabolic syndrome and a risk factor for type 2 diabetes (1). Thus, polymorphisms in genes associated with lipid homeostasis may also influence glycemic control and the development of type 2 diabetes in hyperlipidemic patients. A common polymorphism in the cholesterol ester transfer protein gene (CETP Taq1B, rs708272) was associated in the past with metabolic syndrome (2) and, more recently, with the risk for type 2 diabetes in patients with hyperlipidemia (3). This effect was shown to be modified by another common polymorphism in the gene coding for hepatic lipase (LIPC –G250A, rs2070895) (3). Both genes encode significant components of reverse cholesterol transfer (RCT) in humans and were shown to interact in determining HDL-C levels on multiple occasions in the past (4–6).

Endothelial lipase (EL), the product of the LIPG gene, is another component of RCT which, besides catalyzing the hydrolysis of HDL phospholipids (and, to a lesser extent, triglycerides), binds heparan sulphate proteoglycans to serve as molecular bridges between the surface of endothelial cells, macrophages and hepatocytes on one hand and lipoproteins on the other hand, thus facilitating the physical interaction between various RCT components in situ (7). Rs2000813 is a common LIPG polymorphism that may impact EL's bridging function by virtue of its causing a Thr-to-Ile substitution (Thr111Ile) on its heparan sulfate binding site (8). It was recently associated with microvascular complications and proliferative retinopathy in patients with type 2 diabetes (9,10).

EL is considered a negative regulator of HDL-C in the circulation (11) and has been positively associated with obesity, metabolic syndrome and type 2 diabetes (12), so we considered it worthwhile to probe the effects of rs2000813 on glycated hemoglobin (A1C), blood lipids and the risk for type 2 diabetes in a group of Greek patients with hyperlipidemia, individually and in combination with the rs708272 polymorphism.

Methods

Study population

This is a retrospective cohort study. The participants were all Greek nationals, residents of northern Greece. The study group consisted originally of 175 consecutive patients, newly diagnosed with hyperlipidemia (total cholesterol [TC]>240 mg/dL and/or TG >200 mg/dL, LDL-C>160 mg/dL) in the outpatient clinics of the First Propedeutic Department of Internal Medicine, AHEPA Hospital, Thessaloniki, and of the General Hospital of Goumenissa, Greece, between November 2011 and February 2013. Exclusion criteria were as follows: the use of lipid-lowering drugs or drugs that otherwise affect the blood lipid profile; the use of insulin; a recent episode of infection or myocardial infarction; and a history or current diagnosis of hypothyroidism, hyperthyroidism or kidney or liver disease. Chronic heavy alcohol consumption was defined as the use of >8 units/week for females and >15 units/week for male drinkers. The study was approved by the ethics committee of the Aristotle University of Thessaloniki Medical School. Peripheral blood collection for DNA isolation was done following the informed consent of the patients.

Laboratory analyses

Blood samples were collected from patients after 12 hours of fasting. Plasma lipids (TC, TG, HDL-C) were determined by conventional enzymatic methods by the same type of instrument (Hitachi 912 analyzer, Roche Diagnostics, Indianapolis, Indiana, USA). The Friedewald equation ([LDL-C]=[TC]–[HDL-C]–[TG]/5) was used to calculate plasma LDL-C concentrations, with the exception of 6 patients with TG >400 mg/dL. Serum EL mass was determined at diagnosis for all but 15 patients by using a commercially available sandwich ELISA kit (ABO Swiss, Beijing, China). A1C levels were determined by a standard high-performance liquid chromatography method, using an HA-8121 analyzer (Menarini Diagnostics, Florence, Italy). Genomic DNA was isolated from venous blood by using a commercially available kit (Ron's Blood DNA minikit, Bioron, Ludwigshaften, Germany). The CETP Taq1B and LIPG Thr111Ile polymorphisms were determined by previously established polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) methods, with minor modifications (13,14). Primers were as follows: CETP Taq1B forward: CACTAGCCCAGAGAGAGGAGTGCC, reverse: CTGAGCCCAGCC GCACACTAAC; LIPG forward: GCCTGTAACCCAGTCACTCTGGAG, reverse: CTACATTGGCGTCTTTCTCTCAT. PCR conditions for CETP Taq1B were: 5' at 95°C, 35 cycles of (30'' at 95°C, 30'' at 61.5°C, 45'' at 72°C) and 5' at 72°C; for LIPG Thr111Ile: 5' at 95°C, 35 cycles of (45'' at 95°C, 30'' at 59°C, 30'' at 72°C) and 5' at 72°C. Restriction enzyme digestion was accomplished with Taq1 (10 μ/reaction, 65°C, 4 hours) (Bioron) for the CETP Taq1B polymorphism (B1 allele: 361+74 bp, B2 allele: 535 bp), and NdeI (10 u/ reaction, 37°C, overnight; Thermo Fisher Scientific, Waltham, Massachusetts, USA) for the LIPG Thr111Ile polymorphism (Thr allele: 308 bp, Ile allele: 282+26 bp). DNA fragments were separated in 2% (CETP Taq1B) and 3% (LIPG Thr111Ile) agarose gels and visualized with ethidium bromide staining (Figure 1).

Statistical analyses

Deviation of genotype distributions for the 2 polymorphisms from the Hardy-Weinberg equilibrium was tested with the chi-square goodness-of-fit. The chi-square test of independence was used to analyze the difference in genotype distributions between patients with diabetes and those without. Independent samples t tests were used to compare continuous variables between the 2 subgroups, with the exception of age, where the Mann-Whitney test was used due to the strong deviation from normality of the age distributions. A multiple logistic regression analysis was then used to examine the effect of parameters that differed significantly between

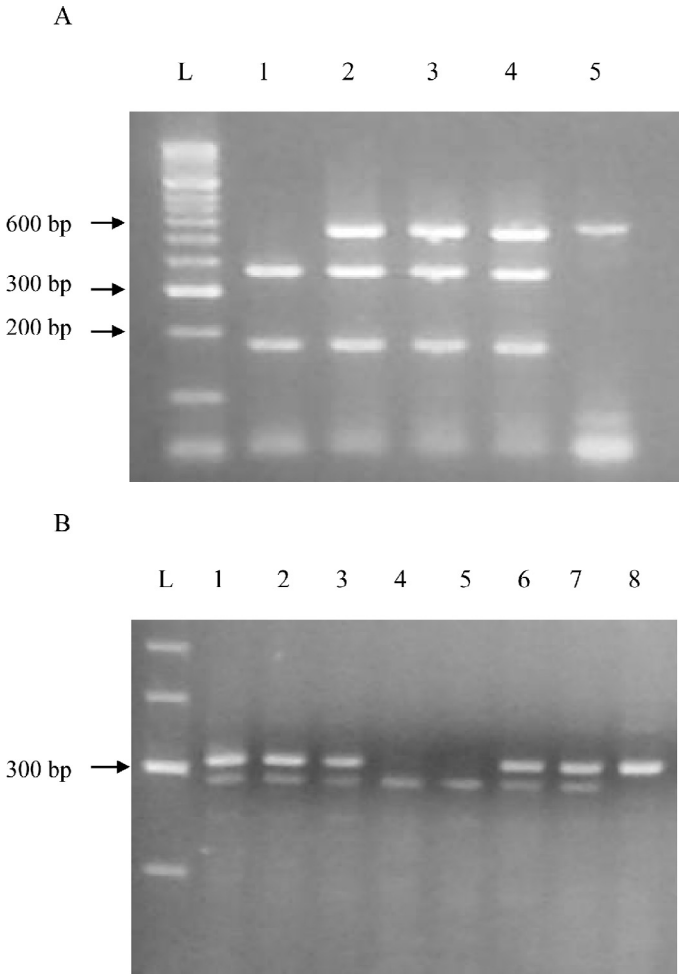


Figure 1. PCR-RFLP of CETP Taq1B (A) and LIPG Thr111Ile (B). A, L=fragment size ladder; 1=B1B1; 2,3,4=B1B2; 5=B2B2. B, L=fragment size ladder; 1,2,3,6,7=ThrIle; 4,5=IleIle; 8=ThrThr.

the 2 subgroups in risk for type 2 diabetes. The effect of univariate and multivariate analyses with plasma lipid concentrations and A1C as dependent variables and CETP Taq1B and LIPG Thr111Ile genotypes, alone or in combination, were run using type III sums of square statistics. Age, sex, BMI, smoking and alcohol consumption were applied as covariates. For the initial multivariate and univariate analyses, p=0.007 was used as the limit of statistical significance, following a Bonferonni correction for 5 continuous variables and 2 polymorphisms. For all other tests, p=0.050 was used. The SPSS (v. 21) statistical package (IBM Corp., Armonk, New York, USA) was used for all calculations.

Results

Study sample

Of the original 175 patients, 75 had been receiving oral antidiabetes treatment in the past 6 months prior to diagnosis of hyperlipidemia or had A1C levels >6.5% and/or fasting glucose >126 mg/dL at diagnosis and were thus considered to have type 2 diabetes. Basic demographic characteristics, average A1C levels, serum EL mass and plasma lipid levels, and the genotype and allele frequency distribution for the 2 polymorphisms, following stratification according to type 2 diabetes, are shown in Table 1. Differences in BMIs, A1C, HDL-C and TG levels occurred in the expected

Table 1
Anthropometric, biochemical and genetic characteristics of the hyperlipidemic patients stratified according to type 2 diabetes

	Non-type 2 diabetes (n=100)	Type 2 diabetes (n=75)	p
Age (years ± SD)	55.3±12.01	60.1±8.70	0.008
Females (%)	49.0	64.0	0.065
Smokers (%)	14.0	14.7	1.000
Alcohol consumption (%)	25.0	6.7	0.001
A1C (% ± SD)	5.4±0.48	7.0±1.00	<0.001
BMI (kg/m^2 ± SD)	29.5±4.41	31.2±4.97	0.019
EL (ng/mL ± SD)	295.9±128.97	330.9±164.61	0.144
TC (mg/dL ± SD)	252.4±48.63	239.8±45.12	0.080
TG (mg/dL ± SD)	237.4±135.92	280.9±172.71	0.064
LDL-C (mg/dL ± SD)	164.7±53.69	149.9±31.22	0.039
HDL-C (mg/dL ± SD)	42.3±10.35	39.1±8.59	0.031
CETP TaqI genotype (n)	99	70	
B1B1 (%)	33.3	31.4	0.963
B1B2 (%)	50.5	51.4	
B2B2 (%)	16.2	17.1	
Allele			
B1 (%)	58.6	57.1	0.788
B2 (%)	41.4	42.9	
Hardy-Weinberg equilibrium	Yes (p=0.923)	Yes (p=0.916)	
LIPG Thr111Ile genotype (n)	98	70	
ThrThr (%)	45.9	45.7	0.056
ThrIle (%)	44.9	32.9	
IleIle (%)	9.2	21.4	
Allele			
Thr (%)	68.4	62.1	0.234
Ile (%)	31.6	37.9	
Hardy-Weinberg equilibrium	Yes (p=0.927)	No (p=0.042)	

A1C, glycated hemoglobin; *BMI,* body mass index; *CETP,* cholesteryl ester transfer protein gene; *EL,* endothelial lipase; *HDL-C,* high density lipoprotein cholesterol; *LDL-C,* low density lipoprotein cholesterol; *LIPG,* endothelial lipase gene; *SD,* standard deviation. *TC,* total cholesterol; *TG,* triglycerides.

directions. Serum EL mass was higher in the subgroup at high risk for type 2 diabetes but not significantly, so CETP Taq1B and LIPG Thr111Ile genotyping failed for 6 and 7 patients, respectively, possibly due to bad quality of their preserved blood samples. Genotype and allele frequencies for the 2 polymorphisms were generally similar to those previously reported for Caucasian populations (9,11,15–17). With the exception of LIPG Thr111Ile in the subgroup with type 2 diabetes, there was no statistically significant deviation from the Hardy-Weinberg equilibrium with respect to either polymorphism studied.

Genotype associations with A1C levels and blood lipids

Tables 2 and 3 summarize the effects of the 2 polymorphisms on average A1C and plasma lipid levels in the 2 subgroups of patients with hyperlipidemia. Among patients without type 2 diabetes (Table 2), the CETP Taq1B polymorphism was clearly associated with HDL-C levels; the B2B2 genotypes displayed the highest values, as expected. In the same subgroup, LIPG Thr111Ile was strongly associated with LDL-C and TC (p<0.001 for either, with the codominant model), whereas neither polymorphism appeared to affect A1C distribution in any significant manner.

However, in the subgroup with type 2 diabetes, a different picture emerged (Table 3). The CETP Taq1B2B2 genotypes displayed significantly lower A1C levels from B1 carriers (p=0.001 for the recessive model), while HDL-C distributions did not differ significantly among the 3 CETP Taq1B genotypes.

We then tested the possibility of an interaction between the 2 polymorphisms in determining A1C and blood lipid levels by multivariate analyses using various ways of stratifying the genotypes (i.e. according to the additive, the dominant and the recessive models). In these analyses, the entire sample was used (patients with and without type 1 diabetes or type 2 diabetes) so as to increase

Table 2
A1C and plasma lipid levels according to CETP Taq1B and LIPG Thr111Ile genotype in hyperlipidemic patients without type 2 diabetes

Plasma variable	CETP Taq1B genotype (99)				LIPG Thr111Ile genotype (98)			
	B1B1 (33)	B1B2 (50)	B2B2 (16)	p	ThrThr (45)	ThrIle (44)	IleIle (9)	p
A1C (% ± SD)	5.3±0.49	5.3±0.46	5.3±0.48	0.987	5.3±0.36	5.4±0.53	5.0±0.53	0.317
TC (mg/dL ± SD)	237.5±47.09	260.2±50.30	260.8±42.36	0.256	267.9±53.78	236.0±40.17	259.9±41.75	0.001
TG (mg/dL ± SD	223.6±106.48	260.5±162.34	186.4±78.21	0.078	237.7±134.28	253.3±142.37	158.1±93.24	0.031
LDL-C (mg/dL ± SD)	152.8±53.12	170.8±55.76	171.5±48.69	0.593	180.6±57.63	145.1±46.90	185.7±40.17	<0.001
HDL-C (mg/dL ± SD)	39.4±8.03	41.0±9.09	52.1±13.18	<0.001	43.4±11. 28	41.4±9.15	43.1±11.69	0.679

A1C, glycated hemoglobin; *BMI,* body mass index; *CETP,* cholesteryl ester transfer protein gene; *HDL-C,* high density lipoprotein cholesterol; *LDL-C,* low density lipoprotein cholesterol; *LIPG,* endothelial lipase gene; *TC,* total cholesterol; *TG,* triglycerides; *SD,* standard deviation.
Note: Age, sex, BMI, smoking and alcohol consumption were used as covariates.

Table 3
A1C and plasma lipid levels according to CETP Taq1B and LIPG Thr111Ile genotype in hyperlipidemic patients with T2D

Plasma variable	CETP Taq1B genotype (70)				LIPG Thr111Ile genotype (70)			
	B1B1 (22)	B1B2 (36)	B2B2 (12)	p	ThrThr (32)	ThrIle (23)	IleIle (15)	p
A1C (% ± SD)	7.1±0.98	7.2±1.04	6.4±0.86	0.005	7.2±1.03	6.7±0.85	7.1±1.20	0.105
TC (mg/dL ± SD)	257.1±50.18	231.9±45.11	231.8±31.10	0.091	235.6±48.58	241.9±38.07	243.5±52.90	0.718
TG (mg/dL ± SD	234.9±73.14	330.2±227.53	223.3±80.75	0.058	313.4±240.36	240.3±98.02	276.4±84.69	0.399
LDL-C (mg/dL ± SD)	169.6±41.26	138.5±34.71	148.9±41.71	0.035	149.2±40.05	152.8±38.29	148.1±45.48	0.990
HDL-C (mg/dL ± SD)	38.8±6.79	38.1±8.91	39.2±10.30	0.758	37.1±7.96	41.5±7.99	37.8±8.54	0.213

A1C, glycated hemoglobin; *CETP,* cholesteryl ester transfer protein gene; *HDL-C,* high-density lipoprotein cholesterol; *LDL-C,* low-density lipoprotein cholesterol; *LIPG,* endothelial lipase gene; *SD,* standard deviation; *TC,* total cholesterol; *TG,* triglycerides.
Note: Age, sex, BMI, smoking and alcohol consumption were used as covariates.

the power of detection. The 2 polymorphisms interacted significantly and consistently in determining HDL-C levels; a weaker interaction was detected with respect to A1C levels (p=0.001 and p=0.015, respectively, with the additive model for both polymorphisms). A graphic representation of those effects is shown in Figure 2.

Genotypes and risk for type 2 diabetes

As shown in Table 1, the endothelial lipase gene (LIPG) 111IleIle genotypes were apparently over-represented in the subgroup with type 2 diabetes compared to the subgroup without type 2 diabetes. When the genotypes were stratified according to the recessive model of inheritance, the comparison of their distribution produced a p value of 0.040 and an OR equal to 2.63 (1.09 to 6.35). However, because the deviation of the subgroup with type 2 diabetes from the Hardy-Weinberg equilibrium rendered application of the chi-square test somewhat risky, a logistic regression analysis was also applied, using the parameters that were significantly different in the 2 subgroups in Table 1 (age, sex, BMI, alcohol consumption, TC, LDL-C, HDL-C, A1C levels and the LIPG genotype distribution (recessive model)] initially proposed as covariates. When all covariates with p>0.10 were removed, the effect of the LIPG genotype on risk for type 2 diabetes became nonstatistically significant but still survived as a trend (p=0.058, OR=3.77 [0.96 to 14.92]).

HDL-C, EL and A1C

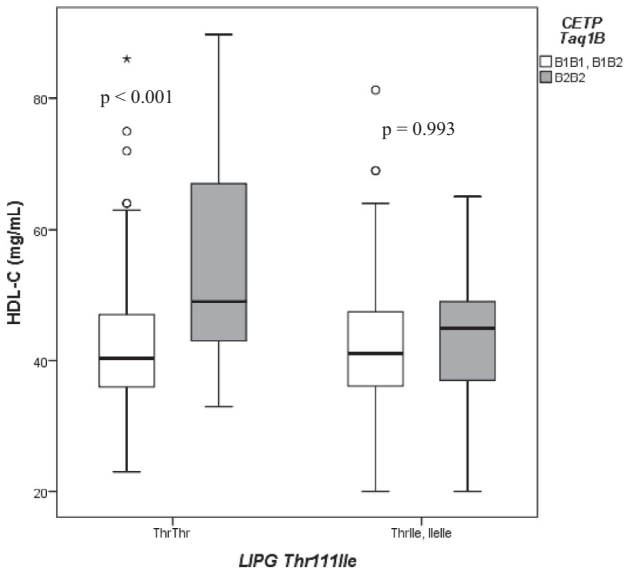
Because the CETP Taq1B polymorphism was associated with both HDL-C and A1C levels, we conducted Pearson correlation tests of these 2 parameters in both subgroups. Our results indicate that the 2 parameters do not correlate in either subgroup (non-type 2 diabetes, r=–0.093; p=0.357; type 2 diabetes: r=–0.005; p=0.965), further supporting that, in our study, CETP Taq1B affected HDL-C and A1C levels independently of each other.

LIPG Thr111Ile had been associated with EL mass (but not EL activity) in the past (18), so we independently assessed that association and also tested the correlation between EL mass and HDL-C and A1C levels in our 2 subgroups. We were able to detect a strong negative correlation between EL mass and HDL-C in both subgroups of patients (r=–0.691; p<0.001, and r=–0.587; p<0.001 for patients with and without type 2 diabetes, respectively), but no correlation between EL mass and A1C levels was found (patients without type 2 diabetes: r=0.197; p=0.057; patients with type 2 diabetes: r=0.016; p=0.898). On the other hand, the association between LIPG Thr111Ile and EL mass was confirmed but was apparently affected by type 2 diabetes status; it is interesting that an unexpected association between CETP Taq1B and EL mass was also observed (Appendix).

Discussion

As early as 1999, Vohl et al (19) reported that the association of the CETP Taq1B2 allele with higher blood HDL-C levels in men was weakened by insulin resistance. Similarly, Heilbronn et al (20) showed that, in women, the same association is significant only for subjects without diabetes with low fasting insulin. According to more recently acquired evidence, CETP Taq1B exerts a protective effect on the development of metabolic syndrome and/or type 2 diabetes (2,3,21). The attenuation of the effect on HDL-C has not been satisfactory explained, whereas that on insulin resistance has been attributed to an increased flux of fatty acids to the liver (2). Our own results are supportive of the above observations because, in our group of Greek treatment-naive patients with hyperlipidemia, 1) the CETP Taq1B2B2 genotype was associated with higher HDL-C values only in the nondiabetic subgroup, and 2) the same genotype was associated with lower A1C levels only in patients with diabetes. In addition, no correlation between A1C and HDL-C levels was detected in our study. Therefore, our findings suggest that the effect of CETP Taq1B on glycemic control is independent of its effect on HDL-C, an effect that was suggested in the past (2). Indeed, on the small, dense, TG-rich HDL particles prevalent in type 2 diabetes, CETP may have an effect not directly reflected by blood HDL-C levels, which could, nevertheless, affect the interactions of HDL recently proposed to underlie modulation of glucose metabolism (22). Others have observed otherwise, however; in a larger study of US men with

A



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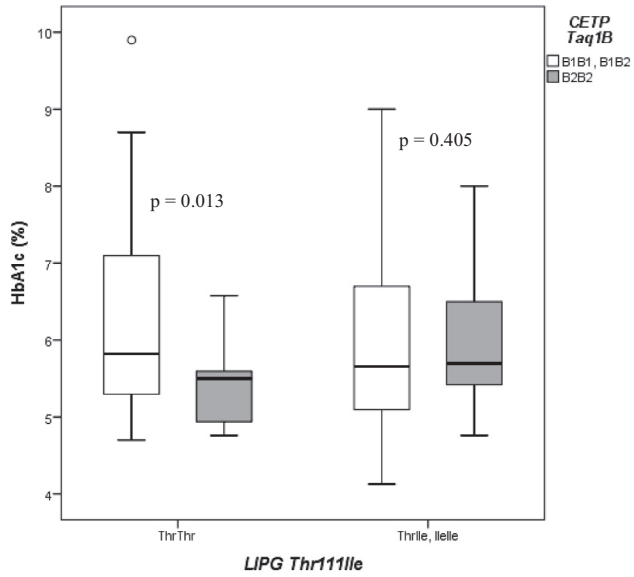

Figure 2. Boxplots of HDL-C and A1C distributions, following stratification according to CETP Taq1B and LIPG Thr111Ile genotype. *Boxes,* interquartile ranges; *vertical lines,* 95% confidence intervals; *horizontal lines,* median values; *small cycles,* outliers; *asterisks,* extreme values.

diabetes, Li et al (23) reported an effect of the CETP Taq1B polymorphism on HDL-C levels of the same magnitude as those in patients without diabetes, whereas no effect on A1C levels was detected. A similar effect on HDL-C levels was recorded in an even larger French study (24). It should be noted, however, that most of the patients with diabetes who participated in those studies were already taking hypoglycemic medication, as opposed to our own, mostly treatment-naive patients. On the other hand, CETP was one of 15 established lipid genes for which evidence of a pleiotropic effect on lipid and glucose-related traits has emerged recently from a much larger study (25).

In contrast to CETP Taq1B, previous evidence suggesting that the effect of LIPG Thr111Ile on plasma lipids is weak and inconsistent (9,18,26) and, on glycemic control, is indirect; this polymorphism has been associated with microvascular complications and proliferative retinopathy in patients with type 2 diabetes (9,10), whereas a positive association was recently found between EL activity and insulin resistance (27). Our own results do not support a strong effect of either serum EL mass or the LIPG Thr111Ile polymorphism on glycemic control, in line with the fact that LIPG was not included in the pleiotropic genes mentioned earlier. On the other hand, this polymorphism appeared to modify the effects of CETP Taq1B on HDL-C and A1C levels, and this constitutes a novel finding.

The relationship of the CETP Taq1B polymorphism with CETP mass and activity is well established (16), but the situation with LIPG Thr111Ile is more complex; the polymorphism does not affect EL activity in vitro, but it may influence EL synthesis in vivo indirectly, as a result of a linkage disequilibrium with another polymorphism (rs34474737), which has been shown to decrease LIPG promoter activity (8,18). Even though EL activity was not determined in this study, our results are suggestive of an effect of the LIPG Thr111Ile polymorphism on the amount of EL present in blood, albeit not a very strong one. The fact that LIPG is upregulated by inflammatory cytokines (28) adds another level of complexity, as does the likely association of LIPG Thr111Ile with EL's bridging function (8). Because the latter is involved in HDL binding by endothelial cells, macrophages and hepatocytes and in LDL binding by endothelial cells and macrophages (7,11), such an effect could manifest itself in altered lipoprotein profiles and cholesterol homeostasis, although directionality would be difficult to predict, given the complexity of the interactions involved and the absence of relevant in vitro data. An effect on EL's bridging function could also be consistent with the apparent modifying effect of LIPG Thr111Ile on the association of CETP Taq1B with HDL-C levels, although the observed effect of CETP Taq1B on EL mass can complicate things. Finally, it could offer an explanation of why the effects of CETP Taq1B, LIPG Thr111Ile and, presumably, other polymorphisms on blood lipids are modulated by type 2 diabetes or the metabolic syndrome. According to Razzaghi et al (8), LIPG Thr111Ile affects a position at the interface of the EL dimer and heparan sulfate proteoglycans. Heparan sulfate is a substrate for heparanase, whose levels are elevated in type 2 diabetes, under the control of glucose and fatty acids (29), and which has been recently implicated in the mobilization of lipoprotein lipase (30). A similar process could conceivably mobilize EL and EL-bound lipoproteins and alter their homeostasis. In support of this hypothesis, digestion of the EL-heparan sulfate proteoglycan complex with heparinase was recently shown to reduce HDL binding to the surface of cultivated endothelial cells (31).

Conclusions

In spite of its obvious limitations, which include a small sample and the lack of data pertinent to other glucose-related traits, our study supports previously reported data that suggest an association of the CETP Taq1B with glycemic control, independent of its effect on HDL-C levels. Moreover, our results suggest a modulating effect of risk for type 2 diabetes and the LIPG Thr111Ile polymorphism on the association of CETP Taq1B with HDL-C and A1C levels, and they offer a tentative explanation for it. No significant independent effect of LIPG Thr111Ile on either A1C levels or risk for type 2 diabetes was observed, although a trend was detected with respect to the latter. Given the strongly suggested importance of fine HDL structure for RCT, anti-inflammatory and antioxidant functions (32), it would be interesting to examine, in future studies, the fine structure of the HDL particles in association with the above genotypes in people with and without diabetes.

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Author Contributions

DA participated in the design of the study, the collection of samples and the analysis of the results, wrote parts of the manuscript, and provided funding; CS participated in the design of the experiments and the collection of samples; KEK contributed to the analysis of the results and the writing of the manuscript; EG performed the PCR experiments and contributed to the analysis of the results; SI oversaw the biochemical analyses; AIH participated in the design and served as coordinator; AG oversaw the genotyping and contributed to the analysis of the results and the writing of the manuscript.

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Appendix

El mass distributions in hyperlipidemic patients following stratification according to CETP Taq1B and LIPG Thr111Ile genotypes and type 2 diabetes

	CETP Taq1B (n)	EL mass (ng/mL±SD)	LIPG Thr111Ile (n)	EL mass (ng/mL±SD)
Non type 2 diabetes	B1B1 (28)	288.1±104.20	ThrThr (43)	294.9±131.77
	B1B2 (50)	321.2±136.94	ThrIle (42)	309.1±130.30
	B2B2 (16)	230.1±124.32	IleIle (8)	232.0±107.99
	p	0.022	p	0.492
Type 2 diabetes	B1B1 (20)	307.9±130.33	ThrThr (30)	405.1±169.81
	B1B2 (34)	379.8±180.50	ThrIle (23)	280.0±101.72
	B2B2 (12)	271.1±141.05	IleIle (14)	281.7±190.56
	p	0.108	p	0.013
Total	B1B1 (48)	296.4±114.90	ThrThr (73)	340.2±157.24
	B1B2 (84)	344.9±157.67	ThrIle (65)	297.0±121.27
	B2B2 (28)	247.6±131.02	IleIle (22)	263.6±164.21
	p	0.003	p	0.049

CETP, cholesteryl ester transfer protein gene; *EL*, endothelial lipase; *LIPG*, endothelial lipase gene; *SD*, standard deviation.