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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