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Polymorphism at the *TRIB1* gene modulates plasma lipid levels: Insight from the Spanish familial hypercholesterolemia cohort study

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Abstract *Background and aims:* rs17321515 SNP has been associated with variation in LDL-C, high density lipoprotein cholesterol and triglycerides concentrations. This effect has never been studied in patients with severe hypercholesterolemia. Therefore, our aims were to assess the association of the rs17321515 (*TRIB1*) SNP with plasma lipids concentrations and anthropometric variables and to explore the interaction between this SNP and some classic risk factors in patients with familial hypercholesterolemia (FH).
Methods and Results: rs17321515 SNP was genotyped in 531 subjects with genetic diagnosis of FH. Homozygous A/A had significantly higher waist circumference compared with G/G subjects ($P = 0.006$) and carriers of the minor allele G ($P = 0.039$). Interestingly, smokers homozygous for the A allele displayed higher plasma triglycerides concentrations ($P = 0.029$), higher VLDL-C levels ($P = 0.023$) and higher TC/HDL-C ratio ($P = 0.035$) than carriers of the minor allele G. In addition, homozygous A/A with the presence of arcus cornealis displayed lower plasma

Abbreviations: TRIB1, Tribbles, drosophila, Homolog of, 1; FH, Familial hypercholesterolemia; CHD, Coronary heart disease; LDLR, Low-density lipoprotein receptor.

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ApoA-I levels ($P = 0.024$) and higher TC/HDL-C ratio ($P = 0.046$) than carriers of the minor allele G.
Conclusions: Smoking status and presence of arcus cornealis modulate the effect of rs17321515 (*TRIB1*) polymorphism on plasma lipids levels in patients with FH. These results could explain the differences in the susceptibility to coronary heart disease in these patients.

Introduction

Familial hypercholesterolemia (FH) is an autosomal codominant inherited disorder of lipoprotein metabolism characterized by high plasma concentrations of low-density lipoprotein cholesterol (LDL-C), deposition of cholesterol in extravascular tissues (xanthomas and arcus cornealis) and increased risk of premature coronary heart disease (CHD). The phenotypic expression of FH is driven, for the most part, by functional mutations in the gene encoding the low-density lipoprotein receptor (LDLR) [1].

It is well known that the phenotypic and clinical expression of cardiovascular disease (CVD) in heterozygous FH patients is highly variable in terms of the age of onset and severity, even for those sharing the same LDLR mutation. This fact supports the notion that pleitropic and non-genetic factors play an important role in disease progression [2,3]. This complexity needs to be properly considered and before genetic tools can be implemented for more precise CHD risk prediction in this high risk population [4,5]. In this regard, risk factors, such as gender and presence of xanthomas and arcus cornealis, may significantly modify the clinical course of the disease. In this context, several studies have shown the relation between corneal lipid deposits and vascular lipid deposits [6,7] and increased atherosclerosis in patients with FH [8]. Moreover, support for epistasis comes from a recent study on 10 candidate loci [9].

The list of candidate genes involved in lipid metabolism is continuously growing [10]. One of the potential new players is the Tribbles, drosophila, Homolog of, 1 (*TRIB1*). Data from human studies have shown that tribbles-1 proteins regulate vascular smooth muscle cell proliferation and chemotaxis, suggesting that these proteins may have an important role in the atherosclerotic process [11—13]. A common SNP (rs17321515 at 8q24) adjacent to the *TRIB1* locus has been associated with variation in LDL-C, high density lipoprotein cholesterol (HDL-C) and triglycerides (TG) concentrations [14—16] and with increased risk for CHD [16].

To date, no studies examining the associations between rs17321515 (*TRIB1*) polymorphism with plasma lipids and other risk factors in patients with severe hypercholesterolemia have been reported. In addition, gene—environment interactions may influence disease progression. Therefore, the aim of the present study was to assess the association of rs17321515 (*TRIB1*) SNP with plasma lipids and anthropometric variables and to identify potential interactions between this SNP and other known risk factors (i.e. gender, smoking, presence of xanthomas and arcus cornealis) in FH subjects.

Methods

Subjects

The study population consisted of 531 unrelated subjects (259 women and 272 men) randomly selected from a Spanish FH longitudinal cohort study, supported by the "Fundación Española Hipercolesterolemia Familiar" (http//www.colesterolfamiliar.com) [17]. All patients included in the study were heterozygous carriers for known functional mutations in the LDLR gene. Patients with familial defective apolipoprotein B disorder were excluded from the analysis.

Demographic data, medical history, lipid lowering therapy, physical examination, pre-treatment lipid profile and family history of CVD were obtained from all patients using standardised protocols [17].

A written informed consent was obtained from all participants before their inclusion in the cohort and the protocol was approved by the ethic committee of the CEIC Fundación Jiménez Díaz (Madrid).

Laboratory methods

Blood samples were obtained after 12 h fasting. Total cholesterol (TC) and TG were determined by enzymatic techniques [18,19]. HDL-C was determined after precipitation with *phosphotungstate* [20]. Very low-density lipoprotein cholesterol (VLDL-C) was determined from plasma TG [21]. ApoA-I and B levels were determined by immunoturbidimetry [22]. LDL-C was calculated using the Friedewald formula [23].

Genetic analyses

Genomic DNA was isolated from whole blood samples using standard methods. All subjects in this study were heterozygous carriers for known mutations in the LDLR gene associated with FH. The genetic diagnosis was made using a DNA-microarray (Progenika, Bilbao, Spain) as previously described [24]. SNP rs17321515 at 8q24 near *TRIB1* was genotyped using a TaqMan® assay with allele-specific probes on the ABIPrism 7900 HT Sequence Detection System (Applied Biosystems) according to routine laboratory protocols [25]. Standard good laboratory practices were undertaken to ensure the accuracy of genotype data, including the inclusion of dummy duplicates.

Statistical analyses

Statistical Package for the Social Sciences (SPSS v 15.0, Chicago, IL, USA) was used for the statistical comparisons.