Journal of Clinical Lipidology (2017) ■, ■–■

Journal of Clinical Lipidology

Original Article

ABCG5/G8 gene is associated with hypercholesterolemias without mutation in candidate genes and noncholesterol sterols

Itziar Lamiquiz-Moneo, MSc*, Lucía Baila-Rueda, PhD, Ana M. Bea, MLT, Rocío Mateo-Gallego, PhD, Sofía Pérez-Calahorra, MSc, Victoria Marco-Benedí, MSc, Antonio Martín-Navarro, PhD, Emilio Ros, PhD, MD, Montserrat Cofán, PhD, José Carlos Rodríguez-Rey, PhD, Miguel Pocovi, PhD, Ana Cenarro, PhD, Fernando Civeira, PhD, MD

Unidad Clínica y de Investigación en Lípidos y Arteriosclerosis, Hospital Universitario Miguel Servet, Instituto de Investigación Sanitaria Aragón (IIS Aragón), CIBERCV, Universidad de Zaragoza, Zaragoza, Spain(Drs Lamiquiz-Moneo, Baila-Rueda, Bea, Mateo-Gallego, Pérez-Calahorra, Marco-Benedí, Martín-Navarro, Cenarro, and Civeira); Unitat de Lipids, Servei d'Endocrinologia i Nutrició, Institut d'Investigacions Biomediques August Pi i Sunyer, Hospital Clinic and CIBER Fisiopatologia de la Obesidad y Nutrición (CIBEROBN), Instituto de Salud Carlos III (ISCIII), Barcelona, Spain(Drs Ros and Cofán); Departamento de Biología Molecular, Facultad de Medicina, Universidad de Cantabria, Instituto de Investigación Valdecilla (IDIVAL), Santander, Cantabria, Spain(Dr Rodríguez-Rey); and Departamento de Bioquímica, Biología Molecular y Celular, Universidad de Zaragoza, Zaragoza, Spain(Dr Pocovi)

KEYWORDS:
Genetic hypercholesterolemia;
ABCG5/G8;
Noncholesterol sterols;
Cholesterol absorption

CONTEXT: Approximately 20% to 40% of clinically defined familial hypercholesterolemia (FH) cases do not show a causative mutation in candidate genes (mutation-negative FH), and some of them may have a polygenic origin.

OBJECTIVE: The aim of this work was to study the prevalence of *ABCG5/G8* genetic variants in mutation-negative FH, as defects in these genes relate to intestinal hyperabsorption of cholesterol and thus *ABCG5/G8* variants could explain in part the mechanism of hypercholesterolemia.

DESIGN, SETTING, AND PATIENTS: We sequenced the *ABCG5/G8* genes in 214 mutation-negative FH and 97 controls. Surrogate markers of cholesterol absorption (5α-cholestanol, β-sitosterol, campesterol, stigmasterol, and sitostanol) were quantified by high-performance liquid chromatography–tandem mass spectrometry in both studied groups.

RESULTS: We found 8 mutation-negative FH patients (3.73%) with a pathogenic mutation in *ABCG5/G8* genes. We observed significantly higher concentration of surrogate markers of cholesterol absorption in mutation-negative FH than in controls. In addition, we found significantly higher concentrations of cholesterol absorption markers in mutation-negative FH with *ABCG5/G8* defects than in mutation-negative, *ABCG5/G8*-negative FH. A gene score reflecting the number of common single

Financial support: This study was supported by grants from the Spanish Ministry of Economy and Competitiveness PI15/01983, PI13/02507, PI12/01321, CIBERCV, CIBEROBN, and Cuenca Villoro Foundation. These projects are co-financed by Instituto de Salud Carlos III and the European Regional Development Fund (ERDF) of the European Union "A way to make Europe."

* Corresponding author. Unidad Clínica y de Investigación en Lípidos y Arteriosclerosis, Hospital Universitario Miguel Servet, Avenida Isabel La Católica 1-3, 50009 Zaragoza, Spain.

E-mail address: itziarlamiquiz@gmail.com

Submitted July 28, 2017. Accepted for publication September 22, 2017.

https://doi.org/10.1016/j.jacl.2017.09.005

nucleotide variants associated with hypercholesterolemia was significantly higher in cases than in controls ($P = .032$). Subjects with a gene score above the mean had significantly higher 5α-cholestanol and stigmasterol than those with a lower gene score.

CONCLUSIONS: Mutation-negative FH subjects accumulate an excess of rare and common gene variations in *ABCG5/G8* genes. This variation is associated with increased intestinal absorption of cholesterol, as determined by surrogate makers, suggesting that these *loci* contribute to hypercholesterolemia by enhancing intestinal cholesterol absorption.

Introduction

Genetic hypercholesterolemias (GHs) are a heterogeneous group of lipid disorders caused by monogenic and polygenic defects and characterized by very high plasma concentrations of total cholesterol (TC) due to increased low-density lipoprotein cholesterol (LDL-C) and high risk of premature coronary heart disease (CHD). Familial hypercholesterolemia (FH) is the most common monogenic GH,[1] with autosomal codominant transmission and with a current estimated prevalence of about 1:200 to 250 in the general population.[2,3] FH is caused by mutations in *LDLR*, the gene coding for the LDL receptor; *APOB*, coding for apolipoprotein (apo) B; *PCSK9*,[4,5] which codes for the enzyme proprotein convertase subtilisin/kexin type 9, or *APOE* genes.[6] In addition, a rare recessive form of FH is also caused by mutations in the *LDLRAP1* gene.[7] However, a causative mutation in candidate genes is not found in approximately 20% to 40% of clinically defined FH cases,[8] suggesting that there are either other as yet unidentified genetic causative *loci* or these cases represent severe polygenic hypercholesterolemia. Actually, affected subjects with clinical FH but without mutations in candidate genes, accumulate some common single-nucleotide variations (SNVs) with a small LDL-C raising effect[9] that do not fully explain the high LDL-C phenotype of these subjects.

Different GWAS have shown that at least 100 *loci* are associated with LDL-C concentration in the population.[10,11] One of these *loci* is *ABCG5/G8*; this gene complex encodes the proteins ABCG5 and ABCG8, which form a heterodimer conveyor located in the membrane of enterocytes and hepatocytes. It has been shown that ABCG5/G8 limits the intestinal absorption of cholesterol and phytosterols and promotes their hepatobiliary secretion. Severe functional mutations in *ABCG5/G8* cause sitosterolemia, a rare autosomal disorder characterized by an increase of phytosterols in blood, up to 30 times greater than normal.[12] In addition, studies have shown that *ABCG5/G8* variation is associated with cholesterol and noncholesterol sterol plasma levels.[13–17] However, the contribution of *ABCG5/G8 loci* variation to mutation-negative FH has not been previously examined.

Considering that mutation-negative FH subjects usually have higher noncholesterol sterol concentrations than other types of GH,[18] probably because of intestinal sterol hyperabsorption, we hypothesized that genetic variations in *ABCG5/G8* are involved in some forms of mutation-negative FH. Hence, the aim of this study was to analyze common and rare mutations in *ABCG5/G8* in a large cohort of mutation-negative FH and assess their association with noncholesterol sterols and LDL-C, to establish the genetic contribution of these *loci* in this type of GH.

Material and methods

Subjects

Unrelated subjects (n = 214), aged 18 to 79 years, with a clinical diagnosis of non-FH GH: LDL-C above the 95th percentile of the Spanish population,[19] triglycerides (TGs) below 200 mg/dL, and familial presentation (at least 1 first-degree relative with the same phenotype) from the Lipid Clinics at Hospital Universitario Miguel Servet, Zaragoza and Hospital Clinic, Barcelona were selected for this study. In all subjects, the absence of a pathogenic mutation in *LDLR*, *APOB*, and *PCSK9* genes was confirmed by the Lipochip platform.[20] Exclusion criteria were the presence of an ε2/ε2 genotype or the p.(Leu167del) mutation in the *APOE* gene. Exclusion criteria were secondary causes of hypercholesterolemia including obesity (body mass index [BMI] >30 kg/m^2), poorly controlled type II diabetes (HbA1c >8%), renal disease with glomerular filtration rate <30 mL/min and/or macroalbuminuria, liver diseases (alanine transaminase >3 times upper normal limit), hypothyroidism (thyroid hormone >6 mIU/L), pregnancy, autoimmune diseases, and treatment with protease inhibitors. Assessment of cardiovascular risk factors, personal and family history of cardiovascular disease, intake of drugs affecting intestinal or lipid metabolism, and anthropometric measurements were performed in all participants. The normolipemic group (n = 97) consisted of healthy, unrelated men and women volunteers aged 18 to 79 years, who underwent a medical examination at the Hospital Miguel Servet of Zaragoza. Exclusion criteria for control subjects were personal or parental history of premature cardiovascular disease or dyslipidemia, current acute illness, or use of drugs that might influence glucose or lipid metabolism. All subjects signed an informed consent to a protocol previously approved by our local ethics committee (Comité Ético de Investigación Clínica of Aragón, Zaragoza, and of Hospital Clínic, Barcelona, Spain).