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Review

Bile acid synthesis precursors in subjects with genetic hypercholesterolemia negative for *LDLR/APOB/PCSK9/APOE* mutations. Association with lipids and carotid atherosclerosis

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ABSTRACT

Some oxysterols are precursors of bile acid synthesis and play an important role in cholesterol homeostasis. However, if they are involved in the pathogeny of genetic hypercholesterolemia has not been previously explored. We have studied non-cholesterol sterol markers of cholesterol synthesis (lanosterol and desmosterol) and oxysterols (7α-hydroxy-4-cholesten-3-one, 24S-hydroxycholesterol and 27-hydroxycholesterol) in 200 affected subjects with primary hypercholesterolemia of genetic origin, negative for mutations in *LDLR, APOB, PCSK9* and *APOE* genes (non-FH GH) and 100 normolipemic controls. All studied oxysterols and cholesterol synthesis markers were significantly higher in affected subjects than controls ($P < 0.001$). Ratios of oxysterols to total cholesterol were higher in non-FH GH than in controls, although only 24S-hydroxycholesterol showed statistical significance ($P < 0.001$). Cholesterol synthesis markers had a positive correlation with BMI, triglycerides, cholesterol and apoB in control population. However, these correlations disappeared in non-FH GH with the exception of a weak positive correlation for non-HDL cholesterol and apoB. The same pattern was observed for oxysterols with high positive correlation in controls and absence of correlation for non-FH GH, except non-HDL cholesterol for 24S-hydroxycholesterol and 27-hydroxycholesterol and apoB for 27-hydroxycholesterol. All non-cholesterol sterols had positive correlation among them in patients and in controls. A total of 65 (32.5%) and 35 (17.5%) affected subjects presented values of oxysterols ratios to total cholesterol above the 95th percentile of the normal distribution (24S-hydroxycholesterol and 27-hydroxycholesterol, respectively). Those patients with the highest levels of 24S-hydroxycholesterol associated an increase in the carotid intima media thickness. These results suggest that bile acid metabolism is affected in some patients with primary hypercholesterolemia of genetic origin, negative for mutations in the candidate genes, and may confer a higher cardiovascular risk. Our results confirm that cholesterol synthesis overproduction is a primary defect in non-HF GH and suggest that subjects with non-FH GH show high levels of oxysterols in response to hepatic overproduction of cholesterol.

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1. Introduction

Autosomal dominant hypercholesterolemias (ADH) are characterized by high levels of low-density lipoprotein (LDL) cholesterol, familial presentation and high risk of premature cardiovascular disease [1]. Most ADH has familial hypercholesterolemia (FH) due to mutations in the *LDLR* gene that encodes for the LDL receptor [2]. Approximately 2–15% of ADH subjects have familial defective apolipoprotein B-100 (FDB) due to mutations in the LDL receptor-binding domain coding region of the *APOB* gene, which encodes for apolipoprotein B-100 [3], or mutations in proprotein convertase subtilisin/kexin type 9 gene (*PCSK9*), a protein involved in the LDL receptor recycling [4]. Recently, a mutation in *APOE* (p.Leu167del) has also been associated with ADH [5,6]. Patients with mutations in these genes present an indistinguishable phenotype and are now included in the FH definition [2]. The genetic cause and pathogenic mechanism of approximately 20–40% of ADH are unknown [7,8], and probably they are a heterogeneous group of diseases including some severe polygenic hypercholesterolemias [9]. For this reason, we named them as non-FH genetic hypercholesterolemias (non-FH GH).

Cholesterol homeostasis is achieved through a highly sophisticated regulation of the uptake, synthesis, esterification and biliary excretion of cholesterol and its derivatives in the body [10]. Oxysterols are oxygenated derivatives of cholesterol that are important as intermediates or end products in cholesterol excretion pathways. The rapid degradation and excretion of oxysterols are facilitated by their physical properties, allowing them to go across lipophilic membranes and to be redistributed in the cell at a much faster rate than cholesterol itself. Important roles with cholesterol turnover, atherosclerosis, apoptosis, necrosis, inflammation, immunosuppression, and the development of gallstones have been described for oxysterols [11–14]. Importantly, oxysterols mediate on cholesterol metabolism to bile acids. The liver nuclear X receptors (LXRs), the liver receptor homologue (LRH) and the hepatocyte nuclear factor (HNF4α) have the ability to bind oxysterols with high affinity [15,16] to produce and secrete bile acids in mice [17]. Important oxysterol ligands for these receptors include: 24S-, 25- and 27- hydroxycholesterol [18] and 3β-hydroxy-5-cholestenoic acid [19].

The classical and quantitatively most important pathway for bile acid synthesis starts with a 7α-hydroxylation of cholesterol via the rate-limiting hepatic cytochrome P-450 enzyme, cholesterol 7α-hydroxylase (CYP7A1) [20]. In addition to the 7α-hydroxylase pathway, there is a bile acid synthesis alternative pathway, starting with the introduction of a hydroxyl group at the terminal methyl group (C27 position) of the steroid side chain [20]. The first step involves the oxidation of cholesterol to 27-hydroxycholesterol by CYP27A1, which is subsequently hydroxylated by oxysterol 7α-hydroxylase (CYP7B1). It has been calculated that 5%–10% of the total conversion of cholesterol into bile acids starts with an extrahepatic 27-hydroxylation [21,22]. Another alternative pathway for bile acid synthesis is through oxidation of cholesterol to 24 (*S*)-hydroxycholesterol. Cholesterol 24-hydroxylase (CYP46A1) is expressed mainly in the brain. There is no synthesis of 24S-hydroxycholesterol from the human liver, hence is a bile acids precursor not related with the hepatic cholesterol synthesis [23].

Pullinger et al. described a non-FH GH kindred carring a loss-of-function mutation in the *CYP7A1* gene, encoding the cholesterol 7α-hydroxylase enzyme, which catalyzes the initial step in cholesterol catabolism and bile acid synthesis. The mutation led to high levels of LDL cholesterol, markedly deficient rate of bile acid excretion, and upregulation of the alternative bile acid pathway [24]. However, the *CYP7A1* gene has not been further associated with FH neither in linkage analysis [25] nor whole exome sequencing of patients without *LDLR/APOB/PCSK9* mutations [26]. Currently, the effect on lipids that can be expected from the accumulation or deficiency of the intermediate metabolites of bile acid synthesis is not clear [18], and it has not been explored so far in subjects with non-FH GH. Given that bile acid formation is a key metabolic pathway, that the association between oxysterols and cholesterol concentration in humans [27] and that a family with a genetic defect in bile acid formation has been described as a cause of genetic hypercholesterolemia [24], we hypothesized that bile acid precursors could be markers of some forms of non-FH GH. In order to better characterize the metabolic abnormalities associated with non-FH GH, we have studied non-cholesterol sterol markers of cholesterol and bile acid hepatic synthesis in a large group of subjects with non-FH GH and normolipemic controls, in which major confounding factors for plasma non-cholesterol sterols were studied. In addition, the non cholesterol sterol profile was analyzed with a sensitive and reliable method by high performance liquid chromatography tandem mass spectrometry (HPLC–MS/MS) [28].

2. Methods

2.1. Study population

Selected subjects (n = 200) were unrelated adults 18–79 years of age with the clinical diagnosis of ADH: LDL cholesterol above the 95th percentile of the Spanish population [29], triglycerides below 200 mg/dL, primary cause and familial presentation (at least one first-degree relative with the same phenotype) from the Lipid Clinic at Hospital Universitario Miguel Servet, Zaragoza, Spain. In all subjects, the presence of functional mutations in *LDLR*, *APOB* and *PCSK9,* and p.Leu167del in *APOE* were ruled out as described below. Secondary causes of hypercholesterolemia including: obesity (body mass index >30 kg/m^2), poorly controlled type 2 diabetes (HbA1c >8%), renal disease with glomerular filtration rate