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Premature atherosclerosis is not systematic in phytosterolemic patients: Severe hypercholesterolemia as a confounding factor in five subjects

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ABSTRACT

Objective: Phytosterolemia is a rare autosomal recessive disorder characterized by dramatically elevated circulating levels of plant sterols (PS). Phytosterolemia is believed to be responsible for severe premature atherosclerosis. The clinical, biological and molecular genetic features of 5 patients with phytosterolemia and transient severe hypercholesterolemia challenge this hypothesis.
Methods: Our patients were referred for suspected homozygous familial hypercholesterolemia. Despite the phenotype, this diagnosis was invalidated and phytosterolemia was confirmed by the identification of mutations in the ABCG5/ABCG8 transporter complex. Plasma PS were analyzed with a mass spectrometric-gas chromatographic procedure. Vascular status was assessed with carotid ultrasonography and completed (for 4 of the 5 patients) with femoral ultrasonography; additional examinations of cardiovascular status included a stress test, determination of coronary calcium score, echocardiography, non-invasive assessment of endothelium-dependent dilatation and coronarography.
Results: The 5 patients displayed markedly elevated levels of both β-sitosterol and campesterol (15–30 fold higher than normal values). However, none displayed significant signs of infraclinical premature atherosclerosis (respectively at the ages of 32, 27, 29, 11 and 11 years). All patients were characterized by very high levels of total (>450 mg/dl) and LDL-cholesterol (>350 mg/dl) at diagnosis which decreased markedly on dietary intervention alone. Treatment with cholestyramine or Ezetimibe ± atorvastatin normalized cholesterol levels, although plasma PS concentrations remained elevated.
Conclusion: The clinical and biological characteristics of our patients, considered together with reports of cases which equally lack CVD, support the contention that the premature atherosclerosis associated with phytosterolemia in some patients may be due at least in part to mechanisms independent of elevated circulating phytosterol levels.

1. Introduction

Phytosterolemia is a rare genetic autosomal recessive disorder caused by abnormalities in the physiological mechanisms that regulate intestinal sterol absorption, and is characterized by dramatic increase in circulating levels of plasma phytosterols including beta-sitosterol, campesterol and stigmasterol. Sitosterol is the most

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abundant phytosterol component, and indeed phytosterolemia is frequently termed sitosterolemia. This disease was first identified in 1974 [1] in 2 sisters of Amish Mennonite background; subsequent examination in a large Amish kindred revealed a pattern of autosomal recessive inheritance [2]. After mapping of the phytosterolemia locus to chromosome 2p21, mutations in two ATP binding cassette (ABC) transporter genes *ABCG5* and *ABCG8* were found to be causative for this disease condition [3]. The *ABCG5* and *ABCG8* genes code for proteins which act as pumps to efflux absorbed phytosterols from enterocytes into the gut and from hepatocytes into the bile ducts. The molecular defect in these transporters results in defective export of plant sterols from enterocytes and hepatocytes and to their elevated levels in plasma and tissues.

In 2004, Sudhop and Von Bergmann estimated that 50–80 cases of confirmed phytosterolemia occurred worldwide [4]. Clinically, phytosterolemia is characterized by marked heterogeneity in phenotype and may involve tendon or tuberoeruptive xanthomata, chronic hemolysis with splenomegaly, arthritis and arthralgias, and premature atherosclerosis. Plant sterols or their oxidized metabolites (oxysterols), or both, may be causally involved in the manifestation of cardiovascular (CV) disease (CVD), possibly via direct effects on the vessel wall or effects on lipoprotein lipoproteins which carry cholesterol such LDL and VLDL particles. However, the pathophysiological mechanisms underlying premature atherosclerosis in some patients presenting homozygous or compound heterozygous phytosterolemia remain unclear. Indeed, the potential causal relationship between plasma plant sterol levels and premature atherosclerosis is far from established, and has recently been brought into question by the EAS Consensus Panel on Plant sterols and stanols [5]. Thus, some cases with markedly elevated levels of plasma plant sterols as revealed by extravascular symptoms have been reported, but in the absence of any sign of premature atheroma [6–8].

We presently report 5 cases of phytosterolemia lacking clinical or subclinical evidence of premature atherosclerosis. The clinical and biological characteristics of our cases, in addition to those in the literature which equally lack CVD, lead us to propose that the premature CVD associated with phytosterolemia in some patients may be due at least in part to mechanisms independent of high plant sterol levels. Specifically, severe hypercholesterolemia is a transient phenomenon frequently observed in patients with biallelic mutations in *ABCG5*/*ABCG8*, and may therefore constitute a major confounder in the vascular disease seen in some phytosterolemic subjects.

2. Material and methods

The clinical features of our cases are based on retrospective data from medical files.

2.1. Recruitment of patients

Four patients were referred to the Clinic for CV Prevention at Pitié-Salpêtrière University Hospital as they displayed very high levels of plasma cholesterol (total cholesterol $>$ 450 mg/dl; LDL-cholesterol $>$ 350 mg/dl) suggestive of homozygous familial hypercholesterolemia. Two were sisters; the other two subjects were unrelated. These 4 subjects are followed clinically by one of the authors (EB). One patient was referred to the University Hospital in Geneva, Switzerland and is followed by NBD. Secondary hypercholesterolemia and particularly hypothyroidism was excluded at the first clinical and biological examination of the patients.

2.2. Sterol measurement

Venous blood samples were obtained after an overnight fast. Samples were withdrawn from the forearm vein into EDTA-treated tubes for plasma and into plain tubes for serum. Plasma samples were rapidly separated by centrifugation at 4 °C, and subsequently analyzed enzymatically for total cholesterol (TC) and HDL-cholesterol (HDL-C) after precipitation of apoB-containing lipoproteins (Roche); LDL-C was calculated with the Friedewald equation. Plasma β-sitosterol and campesterol concentrations were determined on 200 μl aliquots by a mass spectrometric-gas chromatographic procedure adapted from Chevy et al. [9]. Mean plasma levels of phytosterols found in normal subjects are 10 mg/l for sitosterol and 5 mg/l for campesterol respectively [5].

2.3. Genetic analysis

DNA was isolated from whole blood-EDTA samples using the salting out method. The 13 exons of *ABCG5* (NM_022436.2), and the 13 exons of *ABCG8* (NM_022437.2), as well as close flanking intronic sequences, were amplified by PCR. Primer sequences and annealing temperatures are available on request. Direct bidirectional sequencing analysis of the purified PCR product (ExoSAP-IT, GE Healthcare) was carried out on a 3730 DNA analyzer (Applied Biosystems, Life Technologies).

All subjects gave informed consent and DNA analysis was performed in accordance with French bioethics regulations.

2.4. Carotid ultrasonography

In the supine position, with the head turned away from the sonographer and the neck extended with mild rotation, each of the 4 first patients systematically underwent ultrasonography of the extracranial carotid arteries by use of a duplex system (ACUSON Sequoia 512). The protocol involved examination of the right and left common and internal carotid arteries (including bifurcations) with use of a 7.5-MHz scanning frequency in B-mode and a 3.75-MHz frequency in the pulsed-Doppler mode. The approach was posterior, and the sound beam was set perpendicular to the arterial surface. We used a multifrequency configuration (5–8 MHz; access series, mechanical sector scan heads) with a linear array scan head (8L5c) that permitted examination beyond the bifurcation in every case. Intima Media Thickness (IMT) was measured 1 cm from the bifurcation. Three longitudinal measurements of IMT were completed on the right and left CCAs. We used the mean of the 3 right and left longitudinal CCA–IMT measurements in the analysis. All measurements of CCA–IMT were made at sites free of any discrete plaques. If no lesion was detected, the subject was considered normal. The IMT was defined as the distance between the intimal–luminal interface and the medial–adventitial interface. Plaque was defined as an echogenic structure encroaching the vessel lumen with a distinct area 50% greater than the intimal plus media thickness of adjacent sites. The measurement was made perpendicular to the length of the vessel wall.

For Patient 5, non-invasive measurement was performed with a real time B mode ultrasound imager (Vingmed CFM800C system). The right common carotid artery was examined with a 10 MHz vascular probe. IMT and lumen diameter measurements were performed in the arterial segment located 1–2 cm above the bifurcation of the right common carotid artery (CCA). Echographic imaging of the CCA is obtained in the anteroposterior projection with the patient lying supine and the head in the axis.