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A novel mutation of the apolipoprotein A-I gene in a family with familial combined hyperlipidemia

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Abstract

We report a large family in which four members showed a plasma lipid profile consistent with the clinical diagnosis of familial combined hyperlipidemia (FCHL). One of these patients was found to have markedly reduced HDL cholesterol (HDL-C) (0.72 mmol/l) and Apo A-I (72 mg/dl) levels, a condition suggestive of the presence of a mutation in one of the HDL-related genes. The analysis of *APOA1* gene revealed that this patient was heterozygous for a cytosine insertion in exon 3 (c.49–50 ins C), resulting in a frame-shift and premature stop codon at position 26 of pro-Apo A-I (Q17PFsX10). This novel mutation, which prevents the synthesis of Apo A-I, was also found in four family members, including three siblings and the daughter of the proband. Carriers of Apo A-I mutation had significantly lower HDL-C and Apo A-I than non-carriers family members (0.77 ± 0.15 mmol/l vs. 1.15 ± 0.20 mmol/l, $P < 0.005$; 71.4 ± 9.1 mg/dl vs. 134.0 ± 14.7 mg/dl, $P < 0.005$, respectively). Two of the *APOA1* mutation carriers, who were also heavy smokers, had fibrous plaques in the carotid arteries causing mild stenosis (20%). The intimal-media thickness in the two other adult carriers was within the normal range. The other non-carriers family members with FCHL had either overt vascular disease or carotid atherosclerosis at ultrasound examination. This observation suggests that the low HDL-C/low Apo A-I phenotype may result from a genetic defect directly affecting HDL metabolism, even in the context of a dyslipidemia which, like FCHL, is associated with low plasma HDL-C.

Keywords: HDL deficiency; *APOA1* gene mutation; Familial combined hyperlipidemia; Cardiovascular disease

1. Introduction

High density lipoprotein (HDL) metabolism is altered in genetic dyslipidemias due to defects in the metabolism of Apo B-containing lipoproteins.

In familial hypercholesterolemia (FH) abnormalities in HDL metabolism include moderate decrease in plasma HDL cholesterol (HDL-C) and apolipoprotein A-I (Apo A-I) levels. These changes are probably related to an increased fractional catabolic rate of Apo A-I, observed both in homozygous and heterozygous FH [1,2]. Moreover, elevated cholesteryl ester transfer protein (CETP) activity due to an increased number of LDL particles also contributes to the depletion of cholesteryl esters from the plasma HDL pool in FH [3,4]. In hypertriglyceridemia (HTG), due to defective catabolism or overproduction of triglyceride-rich lipoproteins (TG-rich LPs) HDL-C and Apo A-I levels are reduced through various mechanisms: (i) low lipoprotein lipase (LPL) activity results in reduced availability of surface constituents of TG-rich LPs that contribute to HDL formation; (ii) HDL particles, which result from the intravascular lipolysis of TG-enriched HDL, are cleared more rapidly from the circulation; (iii) TG-enriched HDL are intrinsically more unstable in the circulation with Apo A-I loosely bound; (iv) lipolysis of TG-enriched HDL promotes the shedding of Apo A-I from HDL

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particles and its rapid clearance from the circulation [5]. In familial combined hyperlipidemia (FCHL), characterized by elevated serum total cholesterol (TC), elevated triglycerides (TG), or both, and an increased apolipoprotein B (Apo B) concentration, low level of HDL-C and mild reduction of Apo A-I have been reported [6]. In FCHL increased secretion of hepatic Apo B-containing lipoproteins and delayed clearance of TG-rich LPs result in a long residence time for TG-rich LPs in the circulation. Thus TG may be efficiently transferred from TG-rich LPs to HDL and exchanged for HDL cholesteryl esters via the function of CETP. TG-rich HDL particles are preferred substrate for hydrolysis by Hepatic Lipase (HL); this hydrolysis generates more dense HDL particles as well as lipid-poor Apo A-I particles, which are rapidly cleared from the circulation [7,8]. Reduced levels of plasma adiponectin have been recently documented in patients with FCHL. Low plasma levels of adiponectin seems to contribute to the atherogenic lipid profile, including the low HDL-C level [9].

Low levels of HDL-C and Apo A-I are frequently observed in subjects carrying mutations in *APOA1* gene. Overall 70 mutations of this gene, mostly found in heterozygous state, have been reported so far; they are mostly located in the coding region (48 missense, 6 nonsense and 8 frame-shift mutations, and 6 in-frame deletions) (see Refs. [10,11–14]). The mutations which affect the complete translation of Apo A-I mRNA (nonsense and frame-shift mutations) are always associated with reduced levels of Apo A-I and HDL-C. By contrast only one third of the missense mutations and some in-frame deletions are associated with low levels of Apo A-I and HDL-C.

In the present study we report a novel frame-shift mutation of *APOA1* gene discovered in a subject with FCHL, whose plasma lipid profile was characterized by a markedly reduced plasma level of HDL-C and Apo A-I. This mutation was found to segregate with low HDL-C/Apo A-I trait in this FCHL family.

2. Methods

2.1. SM kindred

The proband (II.7 in Fig. 1) was a 39-year-old male who was referred to the lipid clinic for the presence of dyslipidemia and a family history of atherosclerotic cardiovascular disease. The physical examination was negative; more specifically the proband had no corneal opacity, no tendon and planar xanthomas, no enlarged lymph nodes, liver or spleen. He was a heavy smoker and had normal blood pressure. ECG stress test was negative for ischemia but ultrasound carotid examination revealed fibrous-calcific plaques with 20% stenosis. The proband's 76-year-old father had suffered from stroke at the age of 50; at the age of 58 he was found to have a complete obstruction of the right internal carotid artery and 30–40% bilateral obstruction of the common carotid

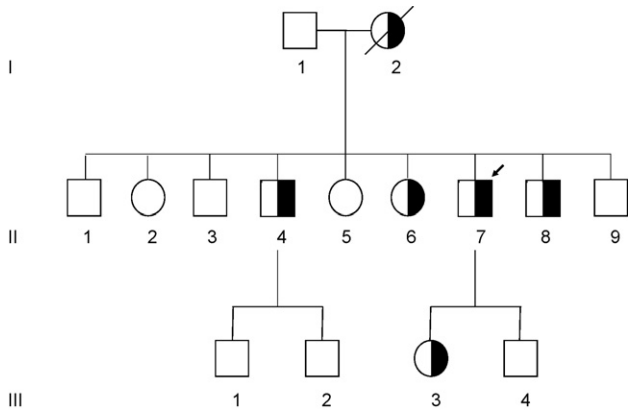

Fig. 1. Pedigree of SM kindred. The proband is indicated with an arrow. The heterozygous carriers of the *APOA1* gene mutation (c.49–50 ins C in exon 3, p.Q17PfsX10) are indicated in black.

arteries. At the age of 74 he was found to have stress-induced myocardial ischemia, as revealed by single-photon emission computed tomography (SPECT). The proband's mother had died at the age of 73 for cerebrovascular and coronary artery diseases. The proband had eight siblings, one of whom (II.3 in Fig. 1) had stage IV peripheral vascular disease and had undergone right femoral-popliteal bypass surgery. Two other siblings (II.2 and II.4) had chronic hepatitis due to HCV infection.

Informed consent was obtained from all subjects investigated. The study protocol was approved by the institutional human investigation committee of each participating institution.

2.2. Biochemical analyses

The plasma concentrations of lipids, including unesterified cholesterol (UC), Apo A-I, Apo B, LpA-I, LpA-I:A-II, as well as HDL particles size and plasma cholesterol esterification rate (CER) were determined as previously described [15]. Serum Apo A-I was detected by immunoblot using an Apo A-I human antibody.

2.3. Sequencing of Apo A-I gene

DNA was extracted from peripheral blood leukocytes [16]. The *APOA1* gene was amplified using the primers previously described [17]. The amplification conditions were: 94 °C for 3 min; 30 cycles at 94 °C for 15 s, 59 °C for 30 s, 72 °C for 3.5 min; 72° for 7 min. The amplification products were sequenced by automatic sequencer CEQ2000 DNA Analysis System (Beckman Coulter, Fullerton, CA).

The cytosine insertion in exon 3 (c.49–50 ins C, Q17PFsX10) was screened by PCR amplification and enzymatic digestion. The PCR primers were: 5′-TCA CCT GGC TGC AAT GAG TGG G-3′ (forward in intron 1) and 5′-TCA ACA TCA TCC CAC AGG CCT TCT-3′ (reverse in intron 3). The conditions were: 94 °C for 3 min; 30 cycles at 94 °C