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A novel ApoA-I truncation (ApoA-I$_{Mytilene}$) associated with decreased ApoA-I production

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ABSTRACT

Objective: We report a novel apolipoprotein (apo) A-I truncation (apoA-I$_{Mytilene}$) due to a heterozygous nonsense mutation (c.718C > T, p.Gln216*) in a 68-year-old male proband with premature coronary heart disease (CHD), corneal arcus, and very low plasma concentrations of HDL cholesterol (HDL-C) and apoA-I. Two family members also had the same mutation. Our objectives were to characterize the kindred and to examine the kinetics of apoA-I, as well as cellular cholesterol efflux capacity in the proband.
Methods: We carried out the kinetic studies using a primed constant infusion of [5,5,5-D$_3$]L-leucine and isotopic enrichment was determined by gas chromatography mass spectrometry in the proband and seven controls with low HDL-C. To assess cellular cholesterol efflux capacity, we used a validated ex vivo system that involved incubation of J774 macrophages with apoB-depleted serum from the proband, five controls with normal HDL-C, and two controls with low HDL-C.
Results: Stable isotope kinetic studies indicated that the proband had an apoA-I production rate (PR) that was 41% lower than the mean PR observed in low HDL-C controls ($n = 7$). The cellular cholesterol efflux capacity assessment showed normalized cholesterol efflux capacity in the proband was decreased by 36% compared to the mean normalized cholesterol efflux capacity of normal controls ($n = 5$).
Conclusions: Our data indicate that this novel heterozygous apoA-I truncation is associated with markedly decreased levels of HDL-C, plasma apoA-I, and apoA-I in large α-1 HDL particles, as well as decreased total cellular cholesterol efflux and decreased apoA-I production.

1. Introduction

Decreased plasma HDL cholesterol (HDL-C) concentrations have been associated with increased coronary heart disease (CHD) risk [1]. Marked HDL deficiency with low apolipoprotein A-I (apoA-I) can be due to apoA-I mutations [2,3]. The major protein constituent of HDL, apoA-I, is crucial for HDL formation, a cofactor for plasma lecithin:cholesterol acyltransferase (LCAT) activation, and cellular cholesterol efflux [4–6]. The *APOA1* gene encodes for a 267 amino acid single peptide chain including a prepropeptide (18 amino acids), and a propeptide (6 amino acids). Mature apoA-I is comprised of 243 amino acids. Structure–function relationships have been established for specific helical regions within apoA-I [7–9]. Therefore, different mutations may have different effects on HDL functions. Some apoA-I mutations result in premature stop codons causing apoA-I truncations. Ten truncations of human apoA-I have been reported previously [10–18]. Prior kinetic studies

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in probands with apoA-I missense or deletion mutations indicated that increased apoA-I fractional clearance was the cause of the HDL deficiency, with no effect on apoA-I production [19–23]. In this study, we report a kindred affected with HDL deficiency and a novel heterozygous apoA-I truncation due to a nonsense mutation, associated with decreased apoA-I production and decreased cellular cholesterol efflux.

2. Methods

2.1. Proband

The proband was a 68 year-old male (Fig. 1, III-13) who presented to the Lipid Clinic at Tufts Medical Center, with a long history of very low HDL-C and premature CHD. His HDL-C levels had ranged from 0.31 to 0.78 mmol/L between the ages of 43–68 years. Prior to statin therapy, the patient had the following plasma lipid concentrations: LDL cholesterol (LDL-C) 4.5 mmol/L, triglyceride (TG) 2.38 mmol/L, and HDL-C 0.49 mmol/L. He had a history of hypertension, but no history of diabetes or smoking. He developed angina at age 54 years. Coronary angiography at that time revealed over 80% stenoses of all major coronary arteries. He underwent triple coronary bypass surgery. He has been followed in our clinic for the past 14 years. His current medications include rosuvastatin 40 mg/day, ezetimibe 10 mg/day, fenofibrate 145 mg/day, omega-3 fatty acid capsules 2 g/day, aspirin 81 mg/day, metoprolol 50 mg/day, and lisinopril 20 mg/day. His recent physical examination revealed: BMI of 28.3 kg/m^2, blood pressure of 130/80 mmHg, a heart rate of 59/min, and normal heart, lung, and neurologic function. There was no evidence of hepatosplenomegaly, xanthomas, or orange tonsils. Popliteal, dorsalis pedis, and posterior tibial pulses in his legs were diminished. An eye examination revealed significant arcus senilis, but no corneal opacification. His most recent plasma lipid concentrations on his current medications are shown in Table 1. The proband's apoA-I gene was sequenced. He also had a stable isotope kinetic study performed to assess apolipoprotein metabolism during atorvastatin therapy. The proband had normal liver, kidney, thyroid function tests, and creatinine kinase levels.

2.2. Family members

His father (II-9) was of Irish origin. He had a myocardial infarction at age 51years, and died of CHD at age 54 years. He had four siblings: two brothers (II-5, II-7) who died of probable CHD at ages 72 and 53 years, a sister (II-8) who died of typhoid fever, and another brother (II-6) who died of cancer. The proband's paternal grandmother (I-2) passed away in a car accident; but she had four male siblings, all of whom died of CHD in their 50s or 60s. No laboratory data was available on these subjects.

The proband's mother (II-10), age 88 years, was of Greek origin. She had a history of a stroke, and had low HDL-C, diabetes, hypertension, and atrial fibrillation. Her laboratory values on the medications pravastatin 10 mg/day, metformin 1000 mg/day, blood pressure medications, and warfarin 5 mg/day are shown in Table 1. Her two sisters (II-11, II-12) died of non-CHD causes. The proband's maternal grandfather (I-3) died of severe hypertension and probable CHD at age 54 years.

One of the proband's brothers (III-14) underwent coronary artery bypass surgery at age 53 years, and had a history of a stroke at age 59 years. His laboratory values at age 64 years on rosuvastatin 40 mg/day are shown in Table 1. One of his sons (IV-18) died suddenly at age 29 years and an autopsy revealed significant coronary heart disease with left main coronary artery stenosis. His wife (III-15) and three other children (IV-19 to 21) were in good health. Another brother of the proband (III-16), age 61 years, had a history of angina and low HDL-C. He underwent coronary angiography, which revealed mild CHD. His laboratory values on rosuvastatin 40 mg/day are shown in Table 1. His wife (III-17) and his three children (IV-22 to 24) were in good health.

2.3. DNA sequencing

DNA was isolated from white blood cells obtained from the proband, his mother and his two brothers. The *APOA1* gene was sequenced using Sanger sequencing methodology using our core sequencing facility. In this study, the protein sequence numbers referred to the mature apoA-I (243 amino acids) and the nucleotide

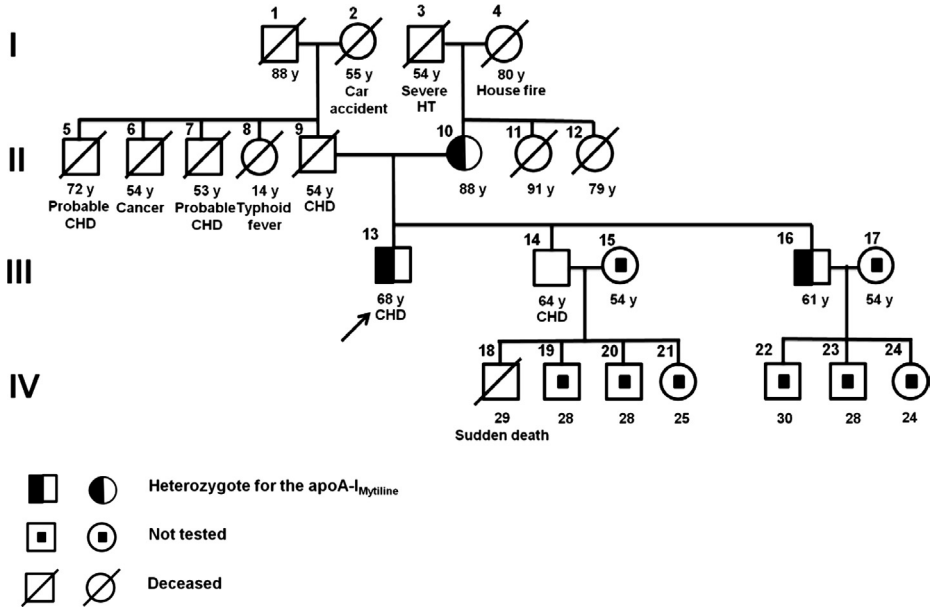

Fig. 1. Pedigree of the proband's family. Arrow indicates the proband. The numbers below the symbols indicate age at the time of this inquiry or at the time of death. The causes of death are described under the symbols.