A Truncated Species of Apolipoprotein B (B-38.7) in a Patient With Homozygous Hypobetalipoproteinemia Associated With Diabetes Mellitus

Ken Ohashi, Shun Ishibashi, Michiyo Yamamoto, Jun-ichi Osuga, Yoshio Yazaki, Susumu Yukawa, Nobuhiro Yamada

Abstract—Familial hypobetalipoproteinemia is caused by mutations in the apolipoprotein (apo) B gene. We identified a 57-year-old woman whose plasma total cholesterol and apoB levels were 2.17 mmol/L and 0.03 g/L, respectively. Separation of plasma lipoproteins by sodium dodecyl sulfate–polyacrylamide gel electrophoresis revealed the absence of apoB-100 and the presence of a faster-migrating form of apoB with an apparent M_r of 195 kDa. Direct sequencing of a polymerase chain reaction–amplified fragment of the patient’s apoB gene DNA revealed a single C→T transition at nucleotide 5472 that converts glutamine 1755 (CAA) to a stop codon (TAA). We predict this novel nonsense mutation of the apoB gene to produce a truncated protein that contains 1754 amino-terminal amino acid residues of apoB-100. We designated this mutant form of apoB apoB-38.7 by following the centile nomenclature of the apoB species. The same mutation was found in both of her children. The proband revealed clinical findings of retinitis pigmentosa, acanthocytosis, and loss of deep tendon reflexes that are characteristic of severe hypobetalipoproteinemia. In addition, the proband had type II diabetes mellitus with nephropathy, anemia, cholelithiasis, hepatic hemangioma, bronchiectasis, and extensive calcification of major arteries including, the celiac, splenic, and renal. In summary, we have found a novel truncated apoB, apoB-38.7, in a patient with an unusual presentation of hypobetalipoproteinemia that includes diabetes mellitus and extensive arterial calcification. (Arterioscler Thromb Vasc Biol. 1998;18:1330-1334.)

Key Words: diabetes mellitus ■ retinitis pigmentosa ■ proteinuria ■ arterial calcification ■ peripheral neuropathy

Familial hypobetalipoproteinemia (HBLP) is a codominant genetic disorder characterized by decreased or absent plasma levels of apolipoprotein (apo) B. (See References 1 and 2 for a review.) Heterozygotes for HBLP have plasma levels of apoB below the fifth percentile and typically are asymptomatic. In Western populations, the frequency of HBLP heterozygotes is estimated between 1 in 500 and 1 in 1000 persons. Homozygotes and compound heterozygotes have extremely low levels of apoB. The clinical phenotype of HBLP homozygotes is highly variable, with severe cases presenting with fat malabsorption, acanthocytosis, retinitis pigmentosa, and neurological complications resulting from intestinal malabsorption of vitamin E. Symptoms observed in some patients with homozygous HBLP are indistinguishable from those of patients with abetalipoproteinemia, a recessive disease arising from mutations in microsomal triglyceride transfer protein (MTP). Since Young and his colleagues demonstrated that mutations in the apoB gene cause HBLP, ~30 mutations have been reported. Most of these mutations are nonsense or frameshift mutations that prevent the translation of the full-length apoB-100 protein. In this report, we describe a Japanese patient with homozygous HBLP caused by a novel mutation in the apoB gene that gives rise to a truncated apoB peptide, apoB-38.7.

Methods

Clinical Data

The proband (K.H.) is a 57-year-old Japanese woman who was referred to Wakayama Medical College for evaluation of a liver mass. Past medical history was significant for childhood asthma, night blindness, and type II diabetes mellitus diagnosed at age 36 years and managed by diet and insulin. At age 48 she had a retinal hemorrhage due to hemorrhagic glaucoma, resulting in right eye blindness despite photoagulation therapy, and at age 54 she developed hemoptysis secondary to bronchiectasis. There was no history of diarrhea or steatorrhea. Family history was unremarkable, although there was no reliable information on consanguinity. She had 2 children, a 32-year-old (A.Y.) and a 30-year-old (H.H.) male, who were the only family members accessible to us for medical investigation.

Physical examination revealed a lean, pale woman 145 cm tall and weighing 37.5 kg. Pulse was 96 bpm and regular; blood pressure was 142/62 mm Hg. Visual acuity was lost in the right eye and 0.06/1.0 in the left with a narrow visual field. Intraocular pressure was 40 mm Hg in the right eye and 15 mm Hg in the left. Retinal pigmentation, hard exudates, and extensive photoagulation scars...
were noted in both optic fundi. Gross hearing loss was noted in the right ear. Examination of the chest and abdomen was unremarkable. Neurological examination revealed paresthesia in both hands, “stocking-glove” type hypohesthesia, absent deep tendon reflexes in the lower extremities, and positive Romberg’s sign. Examination revealed no abnormal pyramidal, cerebellar, or posterior column abnormalities. Laboratory tests revealed anemia (hemoglobin, 7 g/dL) with anacidity, proteinuria (0.6 to 2.3 g/d), mild hyperglycemia (fasting plasma glucose, 120 to 160 mg/dL; stable HbA1c, 6.9%), and reduced creatinine clearance (40 mL/min). Plasma lipid analysis showed that she was moderately hypocholesterolemic, with total cholesterol (TC) levels of 2.17 mmol/L, plasma triglycerides (TGs) of 0.64 mmol/L, HDL cholesterol of 1.99 mmol/L, and plasma apoB of 0.03 g/L.7 Prothrombin time was 10.7 seconds. Plasma lipid analysis of K.H., A.Y., and H.H. are summarized in Table 1. The electroretinogram was flat in both eyes. Abdominal CT scan revealed extensive calcification of the major arteries, including celiac, splenic, common hepatic, renal, and superior mesenteric and a segment of the abdominal aorta (Figure 1). Hepatic hemangioma in the right lobe of the liver was apparent by ultrasonography and MRI. Liver biopsy revealed mild fatty changes. After a 12-hour fast, the lipid contents in each lipoprotein fraction, VLDL (1.006 to 1.063 g/mL), LDL (1.019 to 1.063 g/mL), HDL (1.063 to 1.125 g/mL), and LDL (1.125 to 1.21 g/mL) were isolated by sequential ultracentrifugation as previously described.8 TC, free cholesterol, and TGs were measured enzymatically. For the apolipoprotein analyses, VLDL + IDL (d < 1.019 g/mL), LDL, and HDL (d = 1.063 to 1.21 g/mL) were isolated. After dialysis against a saline solution containing 10 mmol/L phosphate buffer, pH 7.4, 0.15 mol/L NaCl, 1 mmol/L EDTA, and 1 mmol/L PMSF, each lipoprotein fraction was delipidated and subjected to SDS–polyacrylamide gel electrophoresis (PAGE, 3% to 15%). Proteins were visualized by staining with Coomassie brilliant blue R-250. The duodenal mucosa was biopsied endoscopically. Hematoxylin-eosin staining of the biopsied specimen showed no evidence of lipid accumulation in the enterocytes (Figure 2A). Transmission electron microscopy revealed moderate accumulation of fat droplets in the apical cytoplasm of the enterocytes in some sections (magnification ×10 000).

**Table 1. Plasma Concentrations of Lipids, Apolipoproteins, and Vitamin E in the Kindred of K.H.**

<table>
<thead>
<tr>
<th></th>
<th>K.H.</th>
<th>A.Y.</th>
<th>H.H.</th>
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<tbody>
<tr>
<td>TC, mmol/L</td>
<td>2.17</td>
<td>3.21</td>
<td>3.28</td>
</tr>
<tr>
<td>TG, mmol/L</td>
<td>0.64</td>
<td>0.49</td>
<td>0.70</td>
</tr>
<tr>
<td>HDL-C, mmol/L</td>
<td>1.99</td>
<td>1.76</td>
<td>1.84</td>
</tr>
<tr>
<td>apoA-I, g/L</td>
<td>1.33</td>
<td>1.66</td>
<td>1.75</td>
</tr>
<tr>
<td>apoA-II, mg/L</td>
<td>200</td>
<td>370</td>
<td>420</td>
</tr>
<tr>
<td>apoB, mg/L</td>
<td>30</td>
<td>274</td>
<td>268</td>
</tr>
<tr>
<td>apoC-II, mg/L</td>
<td>32</td>
<td>16</td>
<td>21</td>
</tr>
<tr>
<td>apoC-III, mg/L</td>
<td>75</td>
<td>62</td>
<td>67</td>
</tr>
<tr>
<td>apoE, mg/L</td>
<td>35</td>
<td>34</td>
<td>36</td>
</tr>
<tr>
<td>Lp(a), mg/L</td>
<td>56</td>
<td>129</td>
<td>134</td>
</tr>
<tr>
<td>Vitamin E, mg/L</td>
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Lp(a) indicates lipoprotein(a). Apolipoprotein concentrations were measured by the single radial immunodiffusion method.7 Lp(a) concentrations were determined by ELISA. Vitamin E concentrations were determined by high-performance liquid chromatography.

**Other Mutations**

The common cholesteryl ester transfer protein (CETP) gene mutations, an intron 14 splicing defect and an exon 15 missense mutation, Asp442Gly, were evaluated as described.13,14 Other Mutations

**Results**

Lipid contents in each lipoprotein fraction from K.H. are shown in Table 2. Note that LDL contained only 0.12 mmol/L TC. VLDL contained more TC than did LDL.

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**Figure 1.** Abdominal CT scan. Four cross sections are shown (A to D, rostral to caudal). Hepatic hemangioma in the right lobe (A) and calcification of splenic (A and B), celiac (B), common hepatic (B), renal (C), and superior mesenteric (D) arteries and a segment of abdominal aorta (D) are noted.

**Figure 2.** Microscopic examination of biopsied specimen from duodenal mucosa. A, Hematoxylin-eosin staining of microvilli. Enterocytes are apparently normal and do not contain vacuoles, a hallmark of lipid accumulation. B, Electron microscopy of the luminal side of duodenal enterocytes. Electron-lucent lipid droplets are seen in the apical cytoplasm in some sections (magnification ×1 000).

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Most of the TC, 81%, was present in HDL. The HDL2-C/HDL3-C ratio was increased to 3.3.

We sought to identify the cause of K.H.’s hypocholesterolemia. We genotyped the 3' VNTR of the patient’s apoB gene and the CA repeats in intron 10 of the MTP locus. The patient was homozygous for the apoB locus (β37/β37) and heterozygous for the MTP locus. This finding is consistent with the level of plasma TC found in homozygous HBLP and the presence of immunoreactive apoB in plasma (Table 1).

Lipoproteins subjected to SDS-PAGE and Coomassie staining revealed a protein of apparent M, of 195 kDa in VLDL, LDL, and HDL fractions but not in the d=1.21 g/mL fraction (Figure 3). Neither apoB-100 nor apoB-48 was detectable. Based on the apparent molecular weight of the apoB moiety, we estimated a protein size of 1700 to 1800 amino acids. We prepared DNAs by PCR of the apoB gene flanking the site of the predicted mutation. Sequencing of the prepared DNAs revealed a single C->T transition at position 5472 that converts glutamine 1755 (CAA) to a stop codon (TAA) (Figure 4). The calculated centile fraction of the mutant apoB is 38.7%, ie, 1754 of 4536 amino acids. We designated this truncated apoB species apoB38.7, by following the centile nomenclature of the apoB species classification.15 We confirmed that both of the proband’s sons were heterozygous for the identical mutation.

We suspected that the proband had a CETP deficiency based on the elevated plasma HDL-C level (1.99 mmol/L) and the increased HDL2-C/HDL3-C ratio (3.3).16 We genotyped K.H.’s CETP gene for the common CETP gene mutations, an intron 14 splicing defect and an exon 15 missense mutation, Asp442Gly, but neither mutation was detected, supporting the finding of normal plasma CETP activity in K.H.

**Discussion**

The results demonstrate a novel truncation mutation of apoB, apoB-38.7, in the plasma from a patient with homozygous HBLP. ApoB-38.7 is the result of a nonsense mutation at Gln1755 that yields a protein 38.7% the size of apoB-100. Homozygosity for the apoB38.7 allele is supported by homozygosity of the 3' VNTR allele of apoB. Both of the proband’s sons were found to be heterozygous for the mutant allele.
Approximately 30 different mutations have been reported to cause HBLP (see Reference 1 for a review and References 17 through 19), and only 6 were found to be present in the homozygous state.12-18-22 Patients with the most severe phenotype were siblings who were compound heterozygotes for apoB-2 and apoB-9.23 The siblings’ LDL-C levels were undetectable, and they presented with steatorrhea, neurological deficits, and retinitis pigmentosa, a complex of symptoms clinically indistinguishable from that of abetalipoproteinemia. An 8-year-old patient homozygous for apoB-50 also exhibited neurological abnormalities resulting from a nearly complete absence of vitamin E in the plasma.24 Neither neurological symptoms nor retinal degeneration was reported in patients homozygous for apoB-25,26 apoB-27.6,18 apoB-29,12 apoB-39,17 and apoB-45.219 or compound heterozygous for apoB-40/apoB-89.24 In particular, a 48-year-old patient homozygous for apoB-45.2 had a normal plasma level of vitamin E.19 In this context, it is noteworthy that K.H. is homozygous for apoB-38.7 and has neurological deficits, retinal pigmentation, and a flat electroretinogram, the latter 2 of which are indicative of retinitis pigmentosa, despite a normal plasma level of vitamin E (Table 1). Other confounding factors, such as long-standing diabetes mellitus and advancing age, may account for the relatively severe clinical presentation of HBLP in K.H.

In addition to this complex of classic symptoms typical of severe HBLP, K.H. had a wide variety of conditions, such as type II diabetes mellitus, hepatic hemangiomata, cholelithiasis, proteinuria, and a history of retinal hemorrhage, hemoptysis, and arterial calcification. Except for cholelithiasis, which has been reported to be prevalent in the affected members of an apoB-83 kindred,25 the significance of the constellation of these diseases within this patient is currently unknown. These florid complications may be attributable to other recessive mutations. Because HBLP is thought to be protective against atherosclerosis, it is important to note that K.H. had severe calcification of major arteries. One report indicates a relative paucity of coronary artery morbidity and mortality among first-degree relatives of patients with heterozygous HBLP,26,27 furthermore, the virtual absence of atherosclerosis was reported in a 76-year-old subject with HBLP.27

According to her medical history, K.H. had received photocoagulation therapy for “hemorrhagic glaucoma,” which presumably resulted from proliferative diabetic retinopathy. Her renal disease and peripheral neuropathy are also compatible with the clinical picture of diabetic microangiopathy. Because hyperlipidemia is a risk factor for diabetic retinopathy and cholesterol-lowering therapy retards the progression of diabetic nephropathy,28 it is noteworthy that she had advanced complications due to long-standing diabetes.

Another remarkable finding is the unusually high plasma TC and HDL-C levels of K.H. when compared with other HBLP homozygotes. The high HDL2-C/HDL3-C ratio (Table 2)16 and the association with atherosclerosis29 in K.H. are consistent with the clinical features of CETP deficiency, which is a common cause of hyperhighdensitylipoproteinemia in the Japanese.13 However, CETP activity was not decreased, and mutations in either intron 14 or exon 15 of the CETP gene, the most common mutations causing CETP deficiency in the Japanese,13 were not found in the patient. Therefore, it is unlikely that K.H. had CETP deficiency. The associated proteinuria may, at least in part, account for the exceptionally high TC levels in K.H.

We detected apoB-38.7 from VLDL through HDL but not in the d<1.21 g/mL fraction (Figure 3). Previous studies have shown that the buoyant density of the apolipoproteins is largely proportional to the size of apoB between apoB-31 and apoB-37. It has been reported that apoB-31,31 apoB-32,32 and apoB-32.533 are present in the d>1.21 g/mL fraction, but apoB-37 is not,5 suggesting that a portion of the apoB proteins between apoB-32.5 and apoB-37 is critical for the distribution to the d>1.21 g/mL fraction. This finding is in agreement with our observation that apoB-38.7 was not present in the d>1.21 g/mL fraction. ApoB-37,3 apoB-38.9,17 apoB-40,34 and apoB-4620 were reported to be present in HDL, but apoB-39,12 apoB-50,21 and the other species larger than apoB-50 were not, indicating that a portion of the apoB between apoB-37 and apoB-46 is essential for distribution in HDL. This finding is consistent with our observation that apoB-38.7 was distributed in HDL.

In summary, we have found a novel, truncated apoB, apoB-38.7, in a patient with an unusual presentation of HBLP, including diabetes mellitus and extensive arterial calcification.

Acknowledgments

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References


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