Liver Dysfunction and Steatosis in Familial Hypobetalipoproteinemia

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A 32-year-old man presented with increases in serum alanine aminotransferase activity, iron concentration, and transferrin saturation, suggestive of hepatic dysfunction and iron overload. In addition, he had unusually low plasma concentrations of LDL-cholesterol and apolipoprotein (apo) B. Hepatic ultrasonography was consistent with fatty liver. On liver biopsy, marked steatosis and moderate to marked iron deposition were observed. The patient was found to carry the HFE C282Y and H63D mutations, which are associated with hereditary hemochromatosis, and the α₁-antitrypsin PiZ variant. An immunoblot of plasma for apoB showed the presence of a truncated apoB species, indicative of familial hypobetalipoproteinemia. DNA sequence analysis revealed that the patient was heterozygous for the apoB-80.5 (c.11040T>G) mutation. This unique case shows an unusual combination of underlying disorders that could all be contributing to liver dysfunction and fatty liver.

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Familial hypobetalipoproteinemia (FHBL) is a rare codominant disorder characterized by low plasma concentrations of LDL-cholesterol and apolipoprotein (apo) B. More than 50 mutations in the APOB gene leading to FHBL have been reported, most of which encode for a truncated apoB molecule (1–3). Recently we described the first missense mutation that causes hypobetalipoproteinemia, R463W, in an extended Christian Lebanese kindred (4). Heterozygotes for FHBL have apoB concentrations approximately one third of normal, whereas homozygotes can have barely detectable apoB and a range of clinical manifestations, including atypical retinitis pigmentosa and neuromuscular abnormalities (1). These findings are thought to result from low plasma concentrations of fat-soluble vitamin E.

Increased serum transaminases and fatty liver have also been reported in heterozygous FHBL individuals (5, 6). More recently, heterozygous FHBL individuals were shown to have a liver fat content three times higher than normolipidemic control individuals (7, 8). Consistent with these observations, a mouse model of hypobetalipoproteinemia (apoB-38.9) exhibited triglyceride accumulation in hepatocytes (9).

Here we describe a case of FHBL in a 32-year-old man who, in addition to carrying HFE mutations C282Y and H63D, also carries the α₁-antitrypsin PiZ variant. The contribution of these genes to the observed liver dysfunction is discussed.

Case Report

A 32-year-old man presented to his general practitioner with mild persistent right upper quadrant abdominal pain. He had a history of moderate to heavy alcohol intake in the past, but his present alcohol intake was modest and calculated at 10 g/day. His serum γ-glutamyltransferase was within the reference interval. On examination, he appeared well, with a body mass index of 23 kg/m², pulse rate of 70 beats/min, and blood pressure of 110/70 mmHg. His cardiorespiratory and gastrointestinal examinations were normal, but his serum liver function tests showed an isolated increase in alanine aminotransferase activity at 127 U/L, and iron studies showed an increased iron concentration (32 μmol/L) and transferrin saturation (55%), with a high-normal ferritin of 483 μg/L.

Hepatitis serology and anti-mitochondrial antibody were negative. Serum copper and ceruloplasmin were within reference values, but his α₁-antitrypsin concentration (0.9 g/L) was in the low end of the reference interval (0.8–2.0 g/L). He was also noted to have unusually low
plasma concentrations of LDL-cholesterol and apoB (0.4 mmol/L and 0.13 g/L, respectively). APOE genotyping showed that he was homozygous for the wild-type ε3 allele, thus excluding the known impact of the ε2 allele in lowering plasma cholesterol concentrations. Hepatic ultrasonography was consistent with fatty liver. He subsequently underwent a core liver biopsy, which showed severe macrovesicular steatosis and marked hemosiderosis consistent with a history of high alcohol intake (Fig. 1). Immunohistochemical staining for α1-antitrypsin showed clusters of small granules within the cytoplasm of hepatocytes. Genotyping showed that the patient carried the HFE C282Y and H63D mutations in heterozygous form, a finding not considered diagnostic for hereditary hemochromatosis.

The liver pathology did not explain the marked hypocholesterolemia, and he was referred to a Lipid Disorders Clinic for further investigation. There was no evidence for fat malabsorption, malnutrition, or cachexia, making a secondary cause unlikely. Family studies showed that his father (II:1), paternal grandfather (I:1), and youngest brother (III:3) also had marked hypocholesterolemia (Fig. 2 and Table 1). Moreover, hepatic ultrasonography performed in FHBL individual II:1 (the father) was consistent with fatty liver, whereas the results in unaffected individuals II:2 and III:2 were normal.

Extremely low plasma concentrations of LDL-cholesterol and apoB (below the 5th percentile for age and sex) are characteristic of FHBL, a codominant disorder that can be caused by mutations in the APOB gene (2, 3). Approximately 50 mutations in APOB that encode for impaired assembly of triglyceride-rich lipoproteins have been described, most of which lead to the production of a truncated apoB protein. Immunoblotting of plasma was

Fig. 1. Liver biopsy sections showing severe macrovesicular steatosis (A; staining, hematoxylin and eosin; magnification, ×100), heavy hemosiderosis shown by blue pigment (B; Perl’s Prussian Blue; magnification, ×200), and immunohistochemistry for α1-antitrypsin on liver core using toluidine blue counterstain (C; magnification, ×400).

Brown reaction product denotes granule positivity. Periportal hepatocytes show clusters of small (5 µm diameter) granules within the hepatocyte cytoplasm, in some cases displaced by large cytoplasmic fat vacuoles. Nonspecific staining in portal histiocytes is also present.

Fig. 2. Family pedigree.
Patient is indicated by the arrow. Family members with hypocholesterolemia are indicated by □.
performed to screen for a truncated apoB variant, and an extra band corresponding to \(80\%\) of full-length apoB-100 was found (Fig. 3).

To determine the \(\text{APOB}\) mutation responsible, DNA sequence analysis was performed in exon 26 of the \(\text{APOB}\) gene. A single-nucleotide substitution was discovered, which was also found in the other hypocholesterolemic family members (Fig. 2) (10). This mutation, designated c.11040T\(\rightarrow\)G, has been described in another family with hypobetalipoproteinemia and leads to production of apoB-80.5, which is 3652 amino acids in length (11).

Both human in vivo turnover studies and in vitro experiments with carboxyl-terminal truncated mutants have shown a relationship between apoB length and VLDL assembly and secretion (12,13); the shorter the apoB species, the lower the LDL-cholesterol and apoB concentrations will be. On the basis of these studies and the previously reported apoB-80.5 kindred [n=4; mean (SD) plasma apoB concentration, 0.42 (0.06) g/L (11)] a C-terminal loss of \(\sim 20\%\) of the mature protein alone would not be predicted to produce such a marked decrease in plasma apoB as observed in our patient. This suggests that additional factors are impacting hepatic apoB-containing lipoprotein assembly and secretion and, possibly, plasma clearance.

Genotypic analysis of the patient indicated the presence of the \(\alpha_1\)-antitrypsin PiZ variant, consistent with the low-normal serum \(\alpha_1\)-antitrypsin concentration. In addition, all other family members carried the PiZ allele and had low to low-normal concentrations of \(\alpha_1\)-antitrypsin (Table 1). His youngest brother had severe \(\alpha_1\)-antitrypsin deficiency characterized by PiZZ and had died recently of accidental causes.

Table 1. Biochemical and genotyping results.

<table>
<thead>
<tr>
<th>Individual</th>
<th>I:1</th>
<th>II:1</th>
<th>II:2</th>
<th>III:1</th>
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*AST, aspartate aminotransferase; ALT alanine aminotransferase; GGT, \(\gamma\)-glutamyltransferase.

Fig. 3. Western blotting carried out on patients’ plasma using enhanced chemiluminescent detection (A), and sequence of the \(\text{APOB}\) variant found (B).

(A), delipidated plasma samples were subjected to polyacrylamide gel electrophoresis (5% gel) in the presence of 1 g/L sodium dodecyl sulfate and transferred to a nitrocellulose membrane (Bio-Rad) by electroblotting. Enhanced chemiluminescence was carried out according to the manufacturer’s instructions (Amersham Biosciences). Identifiers for the family members (Fig. 2) are indicated above each lane. The patient (lane III:1), as well as having the two typical apoB species, apoB-100 and apoB-48, carried a third species estimated to be 80% of the full-length apoB-100. This apoB variant was also carried by the patient’s brother (lane III:3), father (lane II:1), and grandfather (lane I:1). To identify the mutation responsible, primers were designed to amplify the region of \(\text{APOB}\) thought to harbor the mutation (forward, 5’-ACC CTG GAA CTC TCT CCA TG-3’; reverse, 5’-GGG CAA ATG ATG AAG TTC TCA G-3’). The resulting product was sequenced by the dye-terminator method on an ABI 3100 sequencer (B). A single-nucleotide substitution, c.11040T\(\rightarrow\)G (lower section; wild-type sequence, upper section), was discovered that would create a premature termination codon to form apoB-80.5.
Discussion
We describe a case of FHBL in a 32-year-old man who, in addition to carrying HFE mutations C282Y and H63D, also carries the α1-antitrypsin PiZ variant. We postulate that these three underlying disorders could all be contributing to his liver dysfunction and fatty liver.

Hemochromatosis is a chronic iron overload condition in which excess iron is deposited within tissues. The HFE C282Y allele, associated with hemochromatosis, has a frequency in Australia of 7.2% (14). The H63D allele, although not disease-causing by itself, has been implicated in hemochromatosis when found in conjunction with C282Y (15). Although our patient, who carried both C282Y and H63D, did not have clinical hemochromatosis, marked iron deposition was observed in the liver biopsy.

α1-Antitrypsin is a protease inhibitor produced by the liver, and deficiency can lead to liver and lung disease. More than 70 variants of α1-antitrypsin have been described; the two most common being the S (Glu264Val) and Z (Glu342Lys) alleles. Whereas the S allele has no known clinical significance, the Z allele has a frequency of 1.2% in Australians, and in homozygous individuals decreases α1-antitrypsin concentrations to 10–15% of the values in “normal” M variant carriers (16). Mutant protein accumulates as periodic acid–Schiff-positive, diastase-resistant inclusions in the rough endoplasmic reticulum of hepatocytes, causing damage (17). Although our patient carried a single Z allele, his α1-antitrypsin concentration was low-normal. A brother, individual III:3, was homozygous for the Z variant.

Fatty liver has been reported in FHBL (5). FHBL individuals with a variety of truncated apoB proteins have liver fat content three times higher than unaffected controls (7, 8). FHBL individuals appear to be more susceptible to the effects of adiposity and insulin resistance (7, 8). However, the long-term impact of fatty liver in FHBL is unknown. Although fatty liver is a common occurrence in the general population, it is rare in lean individuals and therefore FHBL should be considered in these cases.

ApoB concentrations in FHBL individuals are typically one third of normal. Our patient had a concentration of 0.13 g/L, which is well below this, given that LDL-cholesterol concentrations correlate to apoB length such that individuals with shorter, more “severe” truncations have lower apoB concentrations (1, 12). However, in this case, the extremely low apoB could reflect the presence of additional liver abnormalities.

In summary, we present a unique case of liver dysfunction with three possible contributors: hemosiderosis associated with HFE C282Y/H63D genotype; α1-antitrypsin PiZ allele; and macrovesicular hepatic steatosis linked to FHBL.

This work was supported by grants from the Royal Perth Hospital Medical Research Foundation, Raine Medical Research Foundation, and the National Heart Foundation of Australia (G 139 1155). P.H.R.B. is a Career Development Fellow of the National Heart Foundation and is supported in part by NIH/National Institute of Biomedical Imaging and Bioengineering Grant EB-001975.

References