Familial hypobetalipoproteinemia: a review

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Abstract We review the genetics and pathophysiology of familial hypobetalipoproteinemia (FHBL), a mildly symptomatic genetically heterogeneous autosomal trait. The minority of human FHBL is caused by truncation-specifying mutations of the APOB gene on chromosome 2. In seven families, linkage to chromosome 2 is absent, linkage is instead to chromosome 3 (3p21). In others, linkage is absent to both APOB and to 3p21. Apolipoprotein B-100 (apoB-100) levels are ~25% of normal, instead of the 50% expected based on the presence of one normal allele due to reduced rates of production. The presence of the truncating mutation seems to have a "dominant recessive" effect on apoB-100 secretion. Concentrations of apoB truncations in plasma differ by truncation but average at ~10% of normal levels. Lipoproteins bearing truncated forms of apoB are cleared more rapidly than apoB-100 particles. In contrast with apoB-100 particles cleared primarily in liver via the LDL receptor, most apoB truncation particles are cleared in renal proximal tubular cells via megalin. Since apoB defects cause a dysfunctional VLDL-triglyceride transport system, livers accumulate fat. Hepatic synthesis of fatty acids is reduced in compensation. Informational lacunae remain about genes affecting fat accumulation in liver, and the modulation of liver fat in the presence apoB truncation defects.—Schonfeld, G. Familial hypobetalipoproteinemia: a review. J. Lipid Res. 2003. 44: 878–883.

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Hypobetalipoproteinemia (HBL) is defined by <5th percentile plasma levels of total cholesterol or LDL cholesterol, or total apolipoprotein B (apoB), i.e., ~150, ~70, and ~50 mg/dl, respectively (1, 2). A number of different factors, such as diet and illness, can produce low apoB levels. Strict vegetarians (vegans) may have total cholesterol levels approaching the 5th percentile (3–5). Intestinal fat malabsorption such as that seen in sprue (chronic pancreatitis), severe liver disease, malnutrition, and hyperthyroidism may produce low apoB and cholesterol levels (6, 7). These are regarded as secondary causes of HBL. Primary causes include abetalipoproteinemia and chylomicron retention disease that segregate in families as autosomal recessive traits, and familial HBL (FHBL) that segregates as an autosomal dominant. Abetalipoproteinemia is due to a variety of genetic defects in microsomal triglyceride transfer protein (MTP), (8–10). The molecular basis of chylomicron retention disease is unknown (11). Both are characterized by an inability to produce chylomicrons in enterocytes, resulting in malabsorption of dietary fat and fat-soluble vitamins that produce severe sequelae in infancy and childhood such as "failure to thrive," anemia, acanthocytosis, ataxia, and retinitis pigmentosa. Early diagnosis and treatment can prevent many of the sequelae. By contrast, the overwhelming majority of subjects with FHBL are simple heterozygotes who are asymptomatic and yet have very low LDL cholesterol and apoB levels. Recently, however, it has been appreciated that a large proportion of subjects have nonalcoholic fatty livers (12–16). The long-term effects of this for health and longevity are unknown. Since the causes of FHBL are unknown in most cases (17), it is useful to identify additional relatives with low levels of LDL or apoB to make the diagnosis of FHBL, and to rule out the secondary causes of HBL. A few FHBL heterozygotes may have loose stools due to partial fat malabsorption, while homozygotes or compound heterozygotes may suffer from severe fat malabsorption, resembling patients with abetalipoproteinemia. As a rule though, even the severe forms of FHBL are less problematic for patients than abetalipoproteinemia (7, 18). Studies in humans, mouse models, and in cultured cells have helped to elucidate the genetic, pathophysiology, and cellular/molecular bases of FHBL.

HUMAN STUDIES

Genetics

In most cases of HBL, the genetic cause(s) are not known (17). The best-characterized cases are due to mutations of the apoB gene. Most are missense and frame-shift mutations specifying the production of truncated proteins (19–21). The truncations are designated according to a centile nomenclature. Thus, the normal protein secreted from liver as...
In fact, levels are closer to a combination of low production rates and rapid clearance (see below). Our group has identified 17 of the truncations reported in the literature.

FHBL appears to be genetically heterogeneous. Recently, we identified seven new families in whom no truncated forms of apoB were detectable in plasma, and FHBL segregated as a Mendelian dominant trait. A genome scan followed by linkage analysis revealed linkage to a susceptibility locus on chromosome 3p21 between markers D3S2407 and D3S1767 (37–39). In a third group of five families, genome scanning and linkage studies ruled out linkage to either chromosome 2 or chromosome 3p21 (G. Schonfeld, unpublished observations).

Metabolism

Three subfamilies of apoB-containing lipoproteins may circulate in the plasmas of subjects heterozygous for apoB truncations longer than apoB-27.6. For example, plasmas of subjects heterozygous for apoB-54.8 contain separate particles bearing apoB-100, apoB-27, and apoB-27.6 (35). ApoB-100 and apoB-48 are found in the population of VLDL-sized particles; however, the vast majority of apoB-100 in plasma circulates in association with LDL-sized particles. Diameters of lipoproteins bearing truncated forms of apoB vary in size directly with the lengths of the truncations, i.e., apoB-39-bearing particles have sizes and densities similar to normal VLDL and LDL. ApoB38.9-bearing particles have sizes intermediate between LDL and HDL, and densities similar to large HDLs (25, 40). Short truncations of apoB transport smaller numbers of triglyceride molecules than do the longer ones.

The average concentrations of apoB-100 in plasmas of heterozygotes would be expected to be ~50% of normal. In fact, levels are closer to ~25% of normal. This is due to production rates (determined in vivo) that are ~25% of normal production rates (41–43). The presence of the various forms of apoB on distinct particles permits concurrent metabolic studies to be performed in vivo to determine the kinetic parameters of apoB-100- and apoB truncation-bearing particles relative to each other in the same person. Levels of apoB truncations vary from about 10–30% of that of apoB-100 in the same person (i.e., ~3–9% of apoB concentration in normal subjects). This is due to a combination of low production rates and rapid clearance rates (44–47); however, the relative importance of production and clearance rates in setting plasma levels depends on the truncation in question. For example, the production rate of apoB-89-containing particles is only 15% lower than that of apoB-100 particles, but their clearance rate is more than twice normal due to the enhanced affinity of the interaction of apoB-89 with the LDL receptor (44). For apoB-75 particles, production is more impaired than for apoB-89, but clearance is still rapid due to enhanced interaction with the LDL receptor (48). The lipoproteins bearing apoB truncations shorter than apoB-75 are cleared very rapidly, mostly by the kidney, mediated by megalin/gp330 receptors located in proximal tubule cells (46, 47).

Hepatosteatosis

As a result of low rates of hepatic production of the normal lipid transporter protein, apoB-100, and the impaired capacities, particularly of short truncations of apoB to transport triglycerides, the VLDL export system for lipids is impaired. This would be expected to result in the accumulation of triglycerides and perhaps other lipid components of VLDL in liver. Indeed, several groups have reported on cases of hepatic steatosis detected by ultrasound or, in rare instances, by liver biopsy (12, 14, 15, 49). Recently, Schonfeld et al. have examined 22 individuals with various truncations of apoB ranging from apoB-4 to apoB-89 and 13 controls, using magnetic resonance spectroscopy (MRS) (16). Results are calculated from energy spectra and represent a precise noninvasive method for quantifying liver fat. The mean value for liver fat in FHBL subjects was five times that of controls (50, 51), suggesting that liver triglyceride contents are elevated. Since MRS as we use it cannot distinguish between the fatty acyl determinants associated with triglycerides, cholesteryl esters, and phospholipids, a theoretical possibility exists that all three of the major lipid moieties are elevated in humans. Indeed, it would not be surprising if all three moieties accumulated in liver since all are necessary for VLDL formation and all are part of VLDL particles; however, livers of mice with apoB truncations accumulate only triglycerides to a significant extent (see below). The differences in liver fat content between FHBL and controls could not be accounted for by caloric intake, dietary composition, indexes of total body fat and abdominal fat, or indexes of glucose tolerance and insulin sensitivity, suggesting that the APOB mutations per se were important contributors to fatty liver.

LESSONS FROM FHBL IN MICE AND CULTURED CELLS

Mice and cells

The production of apoB-containing lipoproteins has been studied in cell cultures using human HepG2 and rat Mc7777 hepatoma cell lines and in primary hepatocytes (52–57). In addition, several mice bearing different truncated forms of apoB have been produced that have helped to elucidate the pathophysiology of FHBL (58–61). Maeda’s group has produced an apoB-81 mouse (62, 63), and Young’s group has produced apoB-83 (59) and apoB-39 mice (59). We have produced an apoB-82-expressing HepG2 cell line
and the limited ability of the apoB-38.9 and -27.6 truncations to ferry triglycerides led us to predict that, just as in humans, our mice too would have fatty livers. Indeed, liver triglycerides were increased 1.5-fold and 3-fold in apoB-38.9/+ and apoB-38.9/38.9 mice, respectively, over age- and sex-matched apoB+/+ wild types (64, 65). Similarly, liver triglycerides of apoB-27.6/+ and apoB-27.6/27.6 mice were increased 3-fold and 5-fold (66). Mean cholesterol contents were elevated by ~4%, but this was not statistically significant. Phospholipid contents were also not significantly elevated. The greater accumulation of triglycerides in the animals bearing the shorter truncation is compatible with the more severe defect in the transport capacity of the shorter truncation. It is not known, however, whether an inverse relationship exists between the lengths of apoB truncations over a wide range of lengths and the amount of fat accumulated in liver.

Feedback inhibition of hepatic triglyceride synthesis

We hypothesized that the APOB defect-induced disturbance of triglyceride transport could result in feedback inhibition of fatty acid and/or triglyceride synthesis in liver. Indeed, fatty acid synthesis measured as [14C]acetate incorporation into triglycerides was decreased in a gene-dose dependent fashion (69). This was accompanied by decreases in hepatic mRNA levels for the transcription factor SREBP-1c that regulates several enzymes in the fatty acid synthetic pathway. The mRNA levels for two of the enzymes measured; fatty acid synthase and steroyl-CoA desaturase-1 were also lower. This feedback would tend to limit the amount of fat accumulated in the face of the APOB mutation-induced defect in the triglyceride export pathway. Thus, the amount of fat accumulated is under the control of several genes; the expression of the genes relative to each other could set hepatic triglyceride levels.

A region between apoB-38.9 and apoB-27.6 that supports embryogenesis

Heterozygous crosses between apoB-38.9/+ × apoB-38.9/+ are expected to yield offspring in the following proportions: 25% apoB-38.9/+, 25% apoB-38.9/38.9, and 50% apoB-38.9/+; however, such crosses in fact yield only ~12% apoB-38.9/38.9 offspring (65). Heterozygous crosses of apoB-27.6/+ yield only 3-4% apoB-27.6/27.6 homozygous offspring (66), similar to the yield of null homogotes (apoB-0/0) in apoB-0/+ × apoB-0/+ crosses (58). Thus, the induction of a null mutation or the apoB-27.6 mutation in mouse apoB results in high degrees of embryonic lethality for homozygotes. By contrast, apoB-38.9/+ × apoB-38.9/+ crosses do yield homozygotes that appear at least grossly normal, but in reduced numbers. This suggests that the first 27.6% of the N-terminal region contains very little, if any, sequence (or structure) able to support embryogenesis, but the next 11.3% (the stretch of sequence between apoB-27.6 and apoB-27.6) does contain such structures. The adequacy of this hypothesis was verified by making apoB-38.9/+ × apoB-27.6/+ crosses. The yields of the resultant compound heterozygotes (apoB-38.9/27.6) were nearly identical to the yields of apoB-38.9 homozygotes, i.e., apoB-38.9 was able to “rescue” apoB-27.6 fetuses (66).
ApoB-38.9 can support atherogenesis

ApoE−/− mice develop florid aortic atherosclerosis even while eating normal low-cholesterol and low-fat mouse chow (70, 71), and are widely used as animal models in studies of atherogenesis. ApoB-containing cholesterol-rich particles are responsible for the atherosclerosis in these animals. To assess whether particles containing apoB-38.9 rather than the normal variants apoB-48 or apoB-100 could support the development of aortic lesions, apoB<sup>38.9/38.9</sup> mice were crossed with apoE<sup>−/−</sup> mice. The resultant doubly homozygous apoB<sup>38.9/38.9</sup>/apoE<sup>−/−</sup> mice developed just as much atherosclerosis as the apoB<sup>++/−</sup>/apoE<sup>−/−</sup> mice did. Thus, the first 38.9% of the N-terminal end of apoB contains sufficient structure for lesion formation.

SUMMARY

In summary, the reported studies have elucidated the genetic bases of some forms of FHBL, and the pathophysiology for the low levels of apoB-containing lipoproteins in humans with FHBL and mice engineered to resemble FHBL. They have also provided new information on some of the functional domains of apoB in lipid transport, embryogenesis, and atherogenesis.

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