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Review

Familial hypobetalipoproteinemia: genetics and metabolism

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Abstract. Familial hypobetalipoproteinemia (FHBL), an autosomal dominant disorder, is defined as <5th percentile LDL-cholesterol or apolipoprotein (apo) B in the plasma. FHBL subjects are generally heterozygous and asymptomatic. Three genetic forms exist: (i) premature stop codon specifying mutations of APOB; (ii) FHBL linked to a susceptibility locus on the chromosome 3p21; and (iii) FHBL linked neither to APOB nor to the chromosome 3p21. In heterozygous apoB-defective FHBL, the hepatic VLDL export system is defective because apoB 100, the product of the normal allele, is produced at ~25% of normal rate, and truncated apoB is cleared too rapidly. The reduced capacity for hepatic triglyceride export increases hepatic fat three-fold. Indexes of adiposity and insulin action are similar to controls. 'Knock-in' mouse models of apoB truncations resemble human FHBL phenotypes. Liver fat in the chromosome 3p21-linked FHBL is normal. Elucidation of the genetic basis of the non-apoB FHBL could uncover attractive targets for lipid-lowering therapy. (See note added in proof.)

Key words. LDL-cholesterol; apoB truncation; genetics; lipoprotein metabolism; non-alcoholic fatty liver.

Definition and clinical presentations

Hypobetalipoproteinemia (HBL) is defined by very low levels (<5th percentile) of total apoB and/or low-density lipoprotein (LDL) cholesterol in plasma [1, 2]. Genetic and environmental factors and various illnesses determine levels of LDL cholesterol and apoB in plasma. Thus, eating a strict vegetarian (vegan) diet [3–5], malnutrition from whatever causes, intestinal fat malabsorption (as in non-tropical sprue, chronic pancreatitis), severe liver disease, and hyperthyroidism may produce low apoB and LDL-cholesterol levels [6, 7]. These are regarded as secondary causes of HBL. Primary causes are due to defects along the very loy density lipoprotein (VLDL)/chylomicron production and secretion pathways, and include abetalipoproteinemia (OMIM 200100) and chylomicron retention disease (OMIM 246700), which segregate in families as autosomal recessive traits, and familial hypobetalipoproteinemia (FHBL), which segregates as an autosomal dominant. Abetalipoproteinemia is due to a variety of genetic defects in microsomal triglyceride transfer protein (MTP), which that facilitates the assembly of lipids with apoB in lipoprotein production [8–10]. Chylomicron retention disease, comprising several clinical sub-syndromes such as Anderson disease (OMIM 607689) and Marinesco-Sjogren syndrome (OMIM 246700), is due to mutations in *SARA2*, a member of the Sar1GTPase genes. Sara2 is one of the proteins of COPII membrane vesicles [11] that may participate in the transport of chylomicron-containing COPII vesicles from the endoplasmic reticulum (ER) to Golgi.

In contrast, the overwhelming majority of subjects with FHBL are simple heterozygotes who are asymptomatic. Although non-alcoholic fatty liver is common among subjects heterozygous for apoB truncations [12–16], the long-term effects of the fatty liver for health and longevity are unknown. A few FHBL heterozygotes may

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have loose stools due to partial fat malabsorption, while homozygotes or compound heterozygotes may suffer from severe fat malabsorption. However, as a rule, even the most severe forms of FHBL are less problematic than patients with abetalipoproteinemia or chylomicron retention disease [7, 17]. Fat malabsorption, when present, is treated by limiting the intake of fats containing long-chain fatty acids and substituting medium-chain triglycerides to furnish fat calories. Fat-soluble vitamins may need to be given as well. These are available in injectable forms or as water-soluble emulsions. The fatty liver has to date not been treated, because it is not clear how and whether this needs to be done. More studies are needed.

Genetics

The molecular-genetic bases are not known in most cases of FHBL [18]. The best-characterized cases are found in families in which the low plasma cholesterol and low apoB phenotype co-segregate with mutations of the APOB gene (chromosome 2) specifying the production of truncated apoB proteins [19–21]. The phenotype has been reproduced in mice engineered to express similar mutations (see below). The truncated proteins are designated according to a centile nomenclature. ApoB 100 is the designation for full-length normal protein secreted from liver as part of VLDL particles, consisting of 4536 amino acid residues [17]. The normal intestinal variant associated with chylomicrons is designated as apoB 48. The same gene directs the production of both apoB 48 and apoB 100. ApoB 48 results from a unique editing of apoB messenger RNA (mRNA) by editosome, that converts codon 2153 to a stop codon [22, 23]. The editosome is present in the intestine of all mammals and in the liver of rodents, but not primates. About 45 different abnormal truncations, as short as apoB 2 and as long as apoB 89, have been reported [14, 15, 24–35]. Null alleles probably result in no translated mRNA [19, 27], and one amino acid substitution has also been reported in one FHBL family [36]. ApoBs shorter than apoB 27.6 are usually absent from plasma due to their rapid clearance (see below).

FHBL appears to be genetically heterogeneous, i.e. not all FHBL cases harbor defects in *APOB*. Recently, we identified seven new FHBL families in whom the HBL phenotype appeared to segregate as a Mendelian dominant trait, yet no truncated forms of apoB were detectable in plasma. A genome scan followed by linkage analysis revealed linkage to a susceptibility locus on the chromosome 3p21, between markers D3S2407 and D3S1767 (~41–47 Mb) [37–39]. In a third group of nine families, genome scanning and linkage studies ruled out linkage to either chromosome 2 or chromosome 3p21 [G. Schonfeld, unpublished, table 1].

Metabolism of ApoB truncations

In normal humans, two forms of apoB may circulate in plasma. ApoB 100, the full-length protein produced in the liver and secreted with VLDL particles, and apoB 48, the product of the same gene, produced in enterocytes and secreted with chylomicron [17, 40]. ApoB truncations shorter than apoB 27.6 have not been detected in plasma, probably because they are too rapidly cleared [41]. But plasmas of subjects heterozygous for apoB truncations longer than apoB 27.6 may contain as many as four sub-families of apoB-containing lipoproteins, because truncations shorter than apoB 48 contain a premature stop codon 5′ from the apoB 48 editing site [42]. Thus, there may be apoB truncation-containing particles from

Table 1. Genetic sub-types of familial hypobetalipoproteinemia.

Subtypes of FHBL	ApoB or LDL levels compared with age- and gender-matched controls	Clinical characteristics	Metabolic changes compared with control	References
Truncation-producing mutations of *APOB*	<30%	asymptomatic, fatty liver, loose stools, fat malabsorption	reduced VLDL apoB pool size, reduced VLDL apoB production rate, reduced VLDL triglyceride production rate	12–14, 16, 19, 40, 46, 47, 51, 54
FHBL linked to the chromosome 3p21	~50%	asymptomatic	Reduced VLDL apoB pool size, elevated VLDL apoB fractional catabolic rate, reduced VLDL triglyceride production rate	40, 48, 54a
FHBL linked neither to APOB nor to chr 3p21	~50%	asymptomatic	unknown	Schonfeld, unpublished

liver and intestine, apoB 100 from the liver and apoB 48 from the intestine. In subjects with truncations longer than apoB 48, e.g. heterozygous subjects with apoB 54.8 heterozygotes, plasmas may contain separate families of particles bearing apoB 100, apoB 48 and apoB 54.8 [35]. ApoB 100 forms particles of VLDL, although the vast majority of apoB 100 is found with LDL particles. Intestinal apoB 48 particles are chylomicrons that vary in size from 40 to 400 nm. Sizes and densities of lipoproteins bearing truncated forms of apoB vary with the lengths of the truncations, i.e. apoB 89-bearing particles have sizes and densities similar to normal VLDL and LDL. ApoB 38.9-bearing particles have sizes intermediate between LDL and high-density lipoprotein (HDL), and densities similar to large HDLs [25, 43]. Short truncations of apoB transport smaller numbers of triglyceride molecules than do the longer ones. The major exception to the rule is apoB 48, which, when assembled in enterocytes as noted, is capable of accommodating even more triglyceride than is apoB100-containing VLDL assembled in the liver, suggesting that the machineries assembling TG-rich particles in the liver and intestine may be different.

One would expect the average concentrations of apoB 100 in plasmas of FHBL heterozygotes to be ~50% of normal subjects, reflecting the activity of one normal allele. However, levels are closer to ~25–30% of the normal [44, 45]. This is because the production rates (determined in vivo using infusions of stable isotope-labeled precursor amino acids) are ~25–30% of those found in normal controls [46–48]. Levels of apoB truncations vary from ~3 to 9 % of the total apoB concentration in normal subjects. This is due to a combination of low production rates and rapid clearance rates of the truncated forms [41, 44, 49, 50]. 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 apoB89-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 interaction of apoB 89 with the LDL receptor [49]. The production of apoB 75 particles is reduced compared with that of apoB 89, but apoB 75 clearance is still rapid due to enhanced interaction with the LDL receptor [51]. Lipoproteins bearing apoB truncations smaller than apoB 70.5 are cleared very fast, mostly by the kidney, mediated by megalin/gp330 receptors located in proximal tubule cells [41, 44]. For all these reasons, the VLDL export system for lipids is impaired in the apoB-defective form of FHBL.

Steatohepatosis in FHBL subjects

The dysfunctional VLDL system would be expected to render the livers of apoB-defective FHBL subjects unusually susceptible to the accumulation of lipids. Indeed, several groups have reported on cases of hepatic steatosis detected by ultrasound or, in rare instances, by liver biopsy [12, 14, 15, 52]. Recently, Schonfeld et al. have examined 33 individuals with various truncations of apoB ranging from apoB 4 to apoB 89, and 32 controls, using magnetic resonance spectroscopy (MRS) a precise non-invasive method for quantifying liver fat [53]. The mean value for liver fat in the FHBL subjects was three to five times that of controls [53, 54]. However, interindividual variability in liver fat was large, and overlap between the FHBL and control groups was considerable. To asses the roles of adiposity and insulin action on liver fat in the FHBL subjects due to apoB defects, we examined indexes of total body fat (body mass index, BMI) and abdominal fat (waist/hip ratio, W/H). Masses of abdominal subcutaneous adipose tissue (SAT), retroperitoneal adipose tissue (RPAT) and intra-abdominal adipose tissue (IPAT) were measured directly by magnetic resonance imaging. The indexes of obesity were similar in the FHBL and control groups. Likewise, indexes of glucose tolerance and insulin sensitivity, measured by oral glucose tolerance test, calculations of glucose and insulin areas under the curve (AUC), and HOMA indexes [16], were similar in both groups. These data suggest that the increased hepatic fat seen in the FHBL subjects was not due to generalized or localized obesity, or general insulin resistance. Rather, the *APOB* mutations per se were important contributors to fatty liver. Furthermore, while correlation coefficients between IPAT and liver fat contents were statistically significant in both groups of subjects, slopes of the regression lines of IPAT on liver fat were significantly greater in the apoB-defective FHBL than in controls. Analogous results were seen with regression of indexes of insulin action on liver fat contents. Thus, for any given degree of adiposity or insulin resistance, livers of the FHBL subjects contained more fat than that of controls. The mechanism(s) by which the size of abdominal adipose tissue or the actions of insulin affect liver fat levels is not clear, but whatever the mechanisms, the defective VLDL export of liver fat imparts a greater propensity for the accumulation of fat in the liver.

Comparable studies of hepatic fat, obesity and insulin action were performed in the subjects with the chromosome 3p21-linked FHBL. These studies demonstrated an important phenotypic difference between the apoB truncation and the 3p21 forms of FHBL. While indexes of abdominal and generalized obesity and insulin action were similar in the apoB-truncation and the 3p21 groups (and controls), mean hepatic fat content of the 3p21 subjects was significantly lower than in the apoB truncation group, and similar to controls [54a]. The differences in phenotypes appear to reflect the different genotypes of the apoB-defective and the 3p21-linked groups.

Animal and cellular models of FHBL

Mice and cells

The synthesis and secretion of apoB-containing lipoproteins have been studied in human HepG2 cells, rat Mc7777 hepatoma cell lines and in primary hepatocytes [55–60]. Mouse models containing different truncated forms of apoB have been produced that have helped to elucidate the pathophysiology of FHBL [61–64]. ApoB 81 [65, 66], apoB 83 [66] and apoB 39 [62] harboring mice are available. We have produced an apoB82-expressing HepG2 cell line [67], and two apoB truncation-harboring mice, apoB 38.9 [68] and apoB 27.6 [69], using targeted homologous recombination and the Cre-loxP system to excise any extraneous genomic sequences.

Lipoprotein metabolism

In contrast with humans, mouse livers produce not only apoB 100 (and apoB truncations in FHBL), but also apo B48. Thus, apoB48-containing lipoproteins in mice arise from both liver and intestine. Our $apoB^{+/38.9}$ and $apoB^{+/27.6}$ mice exhibit hypobetalipoproteinemia, with low plasma levels of cholesterol and apoB. As in human heterozygotes, three sub-families of apoB-containing lipoproteins circulate in FHBL mouse plasma: those containing apoB 100, apoB 48 and the apoB truncation [68, 69]. Plasma levels of apoB 100 and apoB 48 are about equal, but levels of the truncations are greatly diminished [68, 70]. In plasmas of the $apoB^{38.9/38.9}$ and $apoB^{27.6/27.6}$ homozygotes, the only apoB containing lipoproteins circulating are the truncated forms. In vivo and in vito primary hepatocyte studies showed that livers of the $apoB^{+/38.9}$ mice secrete apoB 100 at ~$1/10^{th}$ the rate of apoB 48, and apoB 38.9 in equal-molar amounts with apoB 48. In the $apoB^{+/27.6}$ mice, too, apoB 100 is secreted at ~$1/10^{th}$ the rate of apoB 48, but apoB 27.6 is secreted at ~5-fold the rate of apoB 48 on a molar basis [68, 69]. Since plasma levels of both the apoB truncations are less than levels of apoB 48, the abnormal truncation-containing lipoproteins must be cleared more rapidly than apoB48-containing lipoproteins [71, 72]. The more rapid clearance of the short apoBs is compatible with previous in vivo studies in humans and rabbits [41, 44, 49, 50]. Similarly, although more apoB 48 is secreted than apoB 100, plasma levels of apoB 48 lipoproteins are equal or less than levels of apoB 100 lipoproteins, suggesting that apoB 48 lipoproteins are cleared more rapidly than apoB 100-lipoproteins.

As mentioned, the in vivo production rates of apoB 100 in humans are ~25% of those of normal controls, instead of the expected 50% (see above and [46–48]). Similar 'dominant negative' types of effects on apoB 100 secretion are seen in HepG2 cells engineered to express apoB 82 [67] and mice engineered to express apoB 81 [65]. The $apoB^{38.9/38.9}$ mice bred with $apoB^{100/100}$ mice produce $apoB^{38.9/100}$ offspring. Hepatocyte cultures of these animals synthesize only apoB 38.9 and apoB 100 (no apoB 48), similar to humans. In these mice, apoB 100 is secreted at less than the expected 50% of controls. This dominant negative effect is not due to decreased rates of synthesis but to reduced rates of secretion from cells; i.e. intrahepatocytic rates of degradation are increased [73]. The cellular-molecular mechanism responsible for the enhanced rate of degradation remains to be identified.

Hepatosteatosis in mice

The low rate of synthesis and secretion of normal apoBs, the rapid clearance of truncation-containing lipoproteins, and the limited ability of the apoB 38.9 and apoB 27.6 truncations to ferry triglycerides led to the expectation that the apoB-defective mice would have fatty livers, similarly to humans. Indeed, liver triglycerides were increased 1.5- and 3-fold in $apoB^{+/38.9}$ and $apoB^{38.9/38.9}$ mice, respectively, over age- and sex-matched $apoB^{+/+}$ wild types [67, 68]. Liver triglycerides of the $apoB^{+/27.6}$ and $apoB^{27.6/27.6}$ increase 3- and 5-fold, respectively [69]. Mean hepatic cholesterol or phospholipid contents were 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. But it is not known whether an inverse relationship exists between the lengths of apoB truncations and the amount of fat accumulated in the liver, over a wider range of lengths of apoB.

Another possibility that could contribute to hepatic TG accumulation is reduced activity of the beta-oxidation pathway. Evidence for this is lacking. In fact, mRNA levels for carnitine-palmitoyl transferase, the mediator of the initial rate-limiting step of beta-oxidation, are unchanged in apoB 38.9 and apoB 27.6 mice compared with wild-type littermates [74].

Feedback inhibition of hepatic fatty acid synthesis

The *APOB* defect-induced disturbance of triglyceride transport resulted in feedback inhibition of fatty acid and/or triglyceride synthesis in the liver [75]. This was accompanied by decreases in hepatic mRNA levels for the transcription factor SREBP-1c, which regulates several lipogenic 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. This suggests that the amount of fat accumulated is under the control of several genes, a contention supported by the inter-

strain variation of hepatic triglyceride noted in a survey of 10 inbred strains from the Jackson Laboratory. There was an 8-fold spread of hepatic TG levels between the lowest and highest hepatic TG contents [74].

Structure-function studies of ApoB

The region between apoB 38.9 and apoB 27.6 supports embryogenesis

Heterozygous crosses between $apoB^{+/38.9}$ and $apoB^{+/38.9}$ are expected to yield offspring in the following proportions: 25% apoB $^{+/+}$, 25% $apoB^{38.9/38.9}$ and 50% $apoB^{+/38.9}$. However, yields were only ~12% $apoB^{38.9/38.9}$ homozygotes [68]. Analogous crosses of the $apoB^{+/27.6}$ heterozygotes yielded only 3–4% $apoB^{27.6/27.6}$ offspring [69], similar to the yield of null homozygotes ($apoB^{0/0}$) in $apoB^{0/+}$ X $apoB^{0/+}$ crosses [61]. Thus, 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, $apoB^{+/38.9}$ X $apoB^{+/38.9}$ 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 38.9) does contain such structures. The adequacy of this hypothesis was verified by making $apoB^{+/38.9}$ X $apoB^{+/27.6}$ crosses. The yields of the resultant compound heterozygotes ($apoB^{38.9/27.6}$) were nearly identical to the yields of the apoB 38.9 homozygotes. Thus, apoB 38.9 was able to 'rescue' apoB 27.6 fetuses [69]. It is not clear how the sizes of the apoB truncations are related to embryogenesis. It is possible that the short truncations are not secreted in sufficient quantity, that they are unable to assemble, package and transport sufficient lipids in particles, or that their interactions with fetal structures are impaired so as to hinder the unloading of their lipid loads. Our data suggest that within the lengths examined, the longer the apoB, the more functional it is.

ApoB38.9 contains sufficient structure to form particles that support atherogenesis

$ApoE^{-/-}$ mice develop 'spontaneous' aortic atherosclerosis despite eating mouse chow that contains very low amounts of cholesterol and fat [76, 77]. ApoB-containing cholesterol-rich particles are responsible for the atherosclerosis in these animals. To assess whether particles containing apoB 38.9 could support the development of aortic lesions, $apoB^{38.9/38.9}$ mice were crossed with $apoE^{-/-}$ mice to produce doubly homozygous $apoB^{38.9/38.9}/apoE^{-/-}$ mice. The plasmas of these mice contained apoB 38.9 as the sole form of apoB. Yet they developed just as much aortic atherosclerosis as the $apoB^{+/+}/apoE^{-/-}$ mice. Thus, the first 38.9% of the N-terminal end of apoB contains sufficient structure for the formation of aortic lesions [78].

Future work

Subjects with the 3p21-linked form of FHBL have very low plasma apoB and LDL-cholesterol concentrations. Yet they appear to have no excessive prevalence of fatty liver [54a]. Therefore, molecules mediating low cholesterol levels in 3p21-linked subjects could be attractive targets for lipid-lowering therapy. To this end, elucidation of the genetic basis of 3p21-linked FHBL remains an important task.

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Note added in proof. While this manuscript was under production, data on a novel form of FHBL have been published: Low LDL cholesterol in individuals of African descent resulting from frequent nonsense mutations in PCSK9. Cohen J., Persemlidis A., Kotowski I. K., Graham R., Garcia C. K. and Hobbs H. (2005) Nature Genetics **31:** 161–165.

1 Heiss G., Tamir I., Davis C. E., Tyroler H. A., Rifkand B. M., Schonfeld G. et al. (1980) Lipoprotein-cholesterol distributions in selected North American populations: the lipid research clinics program prevalence study. Circulation **61:** 302–315
2 Contois J. H., McNamara J. R., Lammi-Keefe C. J., Wilson P. W., Massov T. and Schaefer E. J. (1996) Reference intervals for plasma apolipoprotein B determined with a standardized commercial immunoturbidimetric assay: results from the Framingham Offspring Study. Clin. Chem. **42:** 515–523
3 Burslem J., Schonfeld G., Howald M. A., Weidman S. W. and Miller J. P. (1978) Plasma apoprotein and lipoprotein lipid levels in vegetarians. Metabolism **27:** 711–719
4 Sacks F. M., Castelli W. P., Donner A. and Kass E. H. (1975) Plasma lipids and lipoproteins in vegetarians and controls. N. Engl. J. Med. **292:** 1148–1151
5 Burr M. L., Bates C. J., Fehily A. M. and St Leger A. S. (1981) Plasma cholesterol and blood pressure in vegetarians. J. Hum. Nutr. **35:** 437–441
6 (2001) Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation and Treatment of High Blood Cholesterol In Adults (Adult Treatment Panel III). JAMA **285:** 2486–2497
7 Kane J. P. and Havel R. J. (1995) Disorders of the biogenensis and secretion of lipoproteins containing the B apolipoproteins. In: The Metabolic and Molecular Bases of Inherited Disease, vol. 2, pp. 1853–1885, Scriver C. R., Beaudet A. L., Sly W. S. and Valle D. (eds), McGraw-Hill Inc., New York
8 Kayden H. J. (2001) The genetic basis of vitamin E deficiency in humans. Nutrition 17:797–798

9 Berriot-Varoqueaux N., Aggerbeck L. P., Samson-Bouma M. and Wetterau J. R. (2000) The role of the microsomal triglygeride transfer protein in abetalipoproteinemia. Annu. Rev. Nutr. 20:663–697

10 Wetterau J. R., Aggerbeck L. P., Bouma M. E., Eisenberg C., Munck A., Hermier M. et al. (1992) Absence of microsomal triglyceride transfer protein in individuals with abetalipoproteinemia. Science **258:** 999–1001

11 Berriot-Varoqueaux N., Dannoura A. H., Moreau A., Verthier N., Sassolas A., Cadiot G. et al. (2001) Apolipoprotein B48 glycosylation in abetalipoproteinemia and Anderson's disease. Gastroenterology **121:** 1101–1108

12 Tarugi P. and Lonardo A. (1997) Heterozygous familial hypobetalipoproteinemia associated with fatty liver. Am. J. Gastroenterol. **92:** 1400–1402

13 Tarugi P., Lonardo A., Ballarini G., Erspamer L., Tondelli E., Bertolini S. et al. (2000) A study of fatty liver disease and plasma lipoproteins in a kindred with familial hypobetalipoproteinemia due to a novel truncated form of apolipoprotein B (APO B-54.5). J. Hepatol. **33:** 361–370

14 Tarugi P., Lonardo A., Ballarini G., Grisendi A., Pulvirenti M., Bagni A. et al. (1996) Fatty liver in heterozygous hypobetalipoproteinemia caused by a novel truncated form of apolipoprotein B. Gastroenterology **111:** 1125–1133

15 Tarugi P., Lonardo A., Gabelli C., Sala F., Ballarini G., Cortella I. et al. (2001) Phenotypic expression of familial hypobetalipoproteinemia in three kindreds with mutations of apolipoprotein B gene. J. Lipid Res. **42:** 1552–1561

16 Schonfeld G., Patterson B. W., Yablonskiy D. A., Tanoli T. S., Averna M., Elias N. et al. (2003) Fatty liver in familial hypobetalipoproteinemia: triglyceride assembly into VLDL particles is affected by the extent of hepatic steatosis. J. Lipid Res. **44:** 470–478

17 Havel R. J. and Kane J. P. (1995) Structure and metabolism of plasma lipoproteins. In: The Metabolic and Molecular Bases of Inherited Disease, vol. 2, pp. 1853–1885, Scriver C. R., Beaudet A. L., Sly W. S. and Valle D. (ed.), McGraw-Hill, New York

18 Wu J., Kim J., Li Q., Kwok P. Y., Cole T. G., Cefalu B. et al. (1999) Known mutations of apoB account for only a small minority of hypobetalipoproteinemia. J. Lipid Res. **40:** 955–959

19 Schonfeld G. (1995) The hypobetalipoproteinemias. Annu. Rev. Nutr. **15:** 23–34

20 Farese R. V. Jr, Linton M. F. and Young S. G. (1992) Apolipoprotein B gene mutations affecting cholesterol levels. J. Intern. Med. **231:** 643–652

21 Linton M. F., Farese R. V. Jr and Young S. G. (1993) Familial hypobetalipoproteinemia. J. Lipid Res. **34:** 521–541

22 Davidson N. O. and Shelness G. S. (2000) ApolipoproteinB: mRNA editing, lipoprotein assembly and presecretory degradation. Annu. Rev. Nutr. **20:** 169–193

23 Chen L. and Chan L. (1996) Control of apolipoprotein B mRNA editing: implication of mRNA dynamics at various maturation stages. J. Theor. Biol. **183:** 391–407

24 Pulai J. I., Zakeri H., Kwok P. Y., Kim J. H., Wu J. and Schonfeld G. (1998) Donor splice mutation (665 + 1 G_T) in familial hypobetalipoproteinemia with no detectable apoB truncation. Am. J. Med. Genet. **80:** 218–220

25 Krul E. S., Kinoshita M., Talmud P., Humphries S. E., Turner S., Goldberg A. C. et al. (1989) Two distinct truncated apolipoprotein B species in a kindred with hypobetalipoproteinemia. Arteriosclerosis **9:** 856–868

26 Talmud P., King-Underwood L., Krul E., Schonfeld G. and Humphries S. (1989) The molecular basis of truncated forms of apolipoprotein B in a kindred with compound heterozygous hypobetalipoproteinemia. J. Lipid Res. **30:** 1773–1779

27 Welty F. K., Guida K. A. and Andersen J. J. (2001) Donor splice-site mutation (210+1G_C) in the ApoB gene causes a very low level of ApoB-100 and LDL cholesterol. Arterioscler. Thromb. Vasc. Biol. **21:** 1864–1865

28 Welty F. K., Hubl S. T., Pierotti V. R. and Young S. G. (1991) A truncated species of apolipoprotein B (B67) in a kindred with familial hypobetalipoproteinemia. J. Clin. Invest. **87:** 1748–1754

29 Welty F. K., Ordovas J., Schaefer E. J., Wilson P. W. and Young S. G. (1995) Identification and molecular analysis of two apoB gene mutations causing low plasma cholesterol levels. Circulation **92:** 2036–2040

30 Pullinger C. R., Hillas E., Hardman D. A., Chen G. C., Naya-Vigne J. M., Iwasa J. A. et al. (1992) Two apolipoprotein B gene defects in a kindred with hypobetalipoproteinemia, one of which results in a truncated variant, apoB-61, in VLDL and LDL. J. Lipid Res. **33:** 699–710

31 Hardman D. A., Pullinger C. R., Hamilton R. L., Kane J. P. and Malloy M. J. (1991) Molecular and metabolic basis for the metabolic disorder normotriglyceridemic abetalipoproteinemia. J. Clin. Invest. **88:** 1722–1729

32 Young S. G., Pullinger C. R., Zysow B. R., Hofmann-Radvani H., Linton M. F., Farese R. V., Jr. et al. (1993) Four new mutations in the apolipoprotein B gene causing hypobetalipoproteinemia, including two different frameshift mutations that yield truncated apolipoprotein B proteins of identical length. J. Lipid Res. **34:** 501–507

33 Talmud P. J., Converse C., Krul E., Huq L., McIlwaine G. G., Series J. J. et al. (1992) A novel truncated apolipoprotein B (apo B55) in a patient with familial hypobetalipoproteinemia and atypical retinitis pigmentosa. Clin. Genet. **42:** 62–70

34 Talmud P. J., Krul E. S., Pessah M., Gay G., Schonfeld G., Humphries S. E. et al. (1994) Donor splice mutation generates a lipid-associated apolipoproteinB-27.6 in a patient with homozygous hypobetalipoproteinemia. J. Lipid Res. **35:** 468–477

35 Wagner R. D., Krul E. S., Tang J., Parhofer K. G., Garlock K., Talmud P. et al. (1991) ApoB-54.8, a truncated apolipoprotein found primarily in VLDL, is associated with a nonsense mutation in the apoB gene and hypobetalipoproteinemia. J. Lipid Res. **32:** 1001–1011

36 Burnett J. R., Shan J., Miskie B. A., Whitfield A. J., Yuan J., Tran K. et al. (2002) A novel nontruncating APOB gene mutation, R463W, causes familial hypobetalipoproteinemia. J. Biol. Chem. **278:** 13442–13452

37 Pulai J. I., Neuman R. J., Groenewegen A. W., Wu J. and Schonfeld G. (1998) Genetic heterogeneity in familial hypobetalipoproteinemia: linkage and non-linkage to the apoB gene in Caucasian families. Am. J. Med. Genet. **76:** 79–86

38 Yuan B., Neuman R., Duan S. H., Weber J. L., Kwok P. Y., Saccone N. L. et al. (2000) Linkage of a gene for familial hypobetalipoproteinemia to chromosome 3p21.1–22. Am. J. Hum. Genet. **66:** 1699–1704

39 Neuman R. J., Yuan B., Gerhard D. S., Liu K. Y., Yue P., Duan S. et al. (2002) Replication of linkage of familial hypobetalipoproteinemia to chromosome 3p in six kindreds. J. Lipid Res. **43:** 407–415

40 Schonfeld G. (2001) Fatty liver in familial hypobetalipoproteinemia. Arterioscler. Throm. Vasc. Biol. **21:** 656

41 Zhu X. F., Noto D., Seip R., Shaish A. and Schonfeld G. (1997) Organ loci of catabolism of short truncations of apoB. Arterioscler Thromb Vasc Biol **17:** 1032–1038

42 Krul E. S., Tang J., Kettler T. S., Clouse R. E. and Schonfeld G. (1992) Lengths of truncated forms of apolipoprotein B (apoB) determine their intestinal production. Biochem Biophys Res Commun **189:** 1069–1076

43 Groenewegen W. A., Averna M. R., Pulai J., Krul E. S. and Schonfeld G. (1994) Apolipoprotein B-38.9 does not associate with apo[a] and forms two distinct HDL density particle populations that are larger than HDL. J. Lipid Res. **35:** 1012–1025

44 Chen Z., Saffitz J. E., Latour M. A. and Schonfeld G. (1999) Truncated apo B-70.5-containing lipoproteins bind to megalin but not the LDL receptor. J. Clin. Invest. **103:** 1419–1430

45 Chen Z., Fitzgerald R. L., Li G., Davidson N. O. and Schonfeld G. (2003) Hepatic secretion of apolipoprotein B-100 is impaired in hypobetalipoproteinemic mice with an apolipoprotein B-38.9-specifying allele. J. Lipid Res. **45:** 155–163

46 Aguilar-Salinas C. A., Barrett P. H., Parhofer K. G., Young S. G., Tessereau D., Bateman J. et al. (1995) Apoprotein B-100 production is decreased in subjects heterozygous for truncations of apoprotein B. Arterioscler. Thromb. Vasc. Biol. **15:** 71–80

47 Elias N., Patterson B. W. and Schonfeld G. (1999) Decreased production rates of VLDL triglycerides and ApoB-100 in subjects heterozygous for familial hypobetalipoproteinemia. Arterioscler. Thromb. Vasc. Biol. **19:** 2714–2721

48 Elias N., Patterson B. W. and Schonfeld G. (2000) In vivo metabolism of ApoB, ApoA-I and VLDL triglycerides in a form of hypobetalipoproteinemia not linked to the ApoB gene. Arterioscler. Thromb. Vasc. Biol. **20:** 1309–1315

49 Parhofer K. G., Barrett P. H., Bier D. M. and Schonfeld G. (1992) Lipoproteins containing the truncated apolipoprotein, Apo B-89, are cleared from human plasma more rapidly than Apo B-100-containing lipoproteins in vivo. J. Clin. Invest. **89:** 1931–1937

50 Parhofer K. G., Daugherty A., Kinoshita M. and Schonfeld G. (1990) Enhanced clearance from plasma of low density lipoproteins containing a truncated apolipoprotein, apoB-89. J. Lipid Res. **31:** 2001–2007

51 Krul E. S., Parhofer K. G., Barrett P. H., Wagner R. D. and Schonfeld G. (1992) ApoB-75, a truncation of apolipoprotein B associated with familial hypobetalipoproteinemia: genetic and kinetic studies. J. Lipid Res. **33:** 1037–1050

52 Lonardo A., Tarugi P., Ballarini G. and Bagni A. (1998) Familial heterozygous hypobetalipoproteinemia, extrahepatic primary malignancy and hepatocellular carcinoma. Dig. Dis. Sci. **43:** 2489–2492

53 Yablonskiy D. A., Ackerman J. J. and Schonfeld G. (2001) Liver-fat content quantification by MR spectroscopy in patients with apoB truncation-containing lipoproteins. Radiology **221:** 496

54 Schonfeld G., Ackerman J. and Yablonskiy D. (2001) Fatty liver in familial hypobetalipoproteinemia (FHBL). Ateriosclerosis, Thrombosis and Vascular Biology **21:** 656

54a Yue P., Tanoli T., Wilhelm O., Yablonsky D. and Schonfeld G. (2005) Absence of fatty liver in familia hypobetalipoprteinemia linked to chromosone 3p21. Metabolism, in press

55 Davis R. A. and Hui T. Y. (2001) 2000 George Lyman Duff Memorial Lecture: atherosclerosis is a liver disease of the heart. Arterioscler. Thromb. Vasc. Biol. **21:** 887–898

56 Ginsberg H. N. (1995) Synthesis and secretion of apolipoprotein B from cultured liver cells. Curr. Opin. Lipidol. 6:275–280

57 Olofsson S. O., Stillemark-Billton P. and Asp L. (2000) Intracellular assembly of VLDL: two major steps in separate cell compartments. Trends Cardiovasc. Med. **10:** 338–345

58 Gordon D. A. and Jamil H. (2000) Progress towards understanding the role of microsomal triglyceride transfer protein in apolipoprotein-B lipoprotein assembly. Biochim. Biophys. Acta **1486:** 72–83

59 Shelness G. S., Ingram M. F., Huang X. F. and DeLozier J. A. (1999) Apolipoprotein B in the rough endoplasmic reticulum: translation, translocation and the initiation of lipoprotein assembly. J. Nutr. **129:** 456S–462S

60 Tran K., Wang Y., DeLong C. J., Cui Z. and Yao Z. (2000) The assembly of very low density lipoproteins in rat hepatoma McA-RH7777 cells is inhibited by phospholipase A2 antagonists. J. Biol. Chem. **275:** 25023–25030

61 Homanics G. E., Smith T. J., Zhang S. H., Lee D., Young S. G. and Maeda N. (1993) Targeted modification of the apolipoprotein B gene results in hypobetalipoproteinemia and developmental abnormalities in mice. Proc. Natl. Acad. Sci. USA **90:** 2389–2393

62 Kim E., Ambroziak P., Veniant M. M., Hamilton R. L. and Young S. G. (1998) A gene-targeted mouse model for familial hypobetalipoproteinemia. Low levels of apolipoprotein B mRNA in association with a nonsense mutation in exon 26 of the apolipoprotein B gene. J. Biol. Chem. **273:** 33977–33984

63 Raabe M., Kim E., Veniant M., Nielsen L. B. and Young S. G. (1998) Using genetically engineered mice to understand apolipoprotein-B deficiency syndromes in humans. Proc. Assoc. Am. Physicians **110:** 521–530

64 Veniant M. M., Kim E., McCormick S., Boren J., Nielsen L. B., Raabe M. et al. (1999) Insights into apolipoprotein B biology from transgenic and gene-targeted mice. J. Nutr. **129:** 451S–455S

65 Srivastava R. A., Toth L., Srivastava N., Hinsdale M. E., Maeda N., Cefalu A. B. et al. (1999) Regulation of the apolipoprotein B in heterozygous hypobetalipoproteinemic knock-out mice expressing truncated apoB, B81. Low production and enhanced clearance of apoB cause low levels of apoB. Mol. Cell. Biochem. **202:** 37–46

66 Farese R. V. Jr, Garg A., Pierotti V. R., Vega G. L. and Young S. G. (1992) A truncated species of apolipoprotein B, B-83, associated with hypobetalipoproteinemia. J. Lipid Res. **33:** 569–577

67 Srivastava R. A., Srivastava N., Averna M., Cefalu A. B. and Schonfeld G. (1999) Molecular bases of low production rates of apolipoprotein B-100 and truncated apoB-82 in a mutant HepG2 cell line generated by targeted modification of the apolipoprotein B gene. J. Lipid Res. **40:** 901–912

68 Chen Z., Fitzgerald R. L., Averna M. R. and Schonfeld G. (2000) A targeted apolipoprotein B-38.9-producing mutation causes fatty livers in mice due to the reduced ability of apolipoprotein B-38.9 to transport triglycerides. J. Biol. Chem. **275:** 32807–32815

69 Chen Z., Fitzgerald R. L. and Schonfeld G. (2002) Hypobetalipoproteinemic mice with a targeted apolipoprotein (Apo) B-27.6-specifying mutation: in vivo evidence for an important role of amino acids 1254–1744 of ApoB in lipid transport and metabolism of the apoB-containing lipoprotein. J. Biol. Chem. **277:** 14135–14145

70 Chen Z., Fitzgerald R. I., Averna M. and Schonfeld G. (2001) Despite hypersecretion of apolipoproteinB-27.6, fatty livers occur in mice with a targeted mutation of the apoB27.6 allele. Arterioscler. Throm. Vasc. Biol. **21:** 669

71 Welty F. K., Lichtenstein A. H., Barrett P. H., Dolnikowski G. G., Ordovas J. M. and Schaefer E. J. (1997) Decreased production and increased catabolism of apolipoprotein B-100 in apolipoprotein B-67/B-100 heterozygotes. Arterioscler. Thromb. Vasc. Biol. **17:** 881–888

72 Welty F. K., Lichtenstein A. H., Barrett P. H., Dolnikowski G. G., Ordovas J. M. and Schaefer E. J. (1997) Production of apolipoprotein B-67 in apolipoprotein B-67/B-100 heterozygotes: technical problems associated with leucine contamination in stable isotope studies. J. Lipid Res. **38:** 1535–1543

73 Chen Z., Fitzgerald R. L., Li G., Davidson N. O. and Schonfeld G. (2004) Hepatic secretion of apoB-100 is impaired in hypobetalipoproteinemic mice with an apoB-38.9-specifying allele. J. Lipid Res. **45:** 155–163

74 Lin X., Yue P., Chen Z. and Schonfeld G. (2004) Hepatic triglyceride contents are genetically determined in mice: results of a strain survey. Am. J. Physiol. Gastrointest. Liver Physiol. [Epub ahead of print]

75 Lin X., Schonfeld G. and Chen Z. (2001) Molecular adaptation of the fatty acid synthetic pathway to the presence of fatty livers in gene-targeted mice producing apolipoprotein B-39.8. Arterioscler. Throm. Vasc. Biol. **21:** 661

76 Dansky H. M., Charlton S. A., Sikes J. L., Heath S. C., Simantov R., Levin L. F. et al. (1999) Genetic background determines the extent of atherosclerosis in ApoE-deficient mice. Arterioscler. Thromb. Vasc. Biol. **19:** 1960–1968

77 Calleja L., Paris M. A., Paul A., Vilella E., Joven J., Jimenez A. et al. (1999) Low-cholesterol and high-fat diets reduce atherosclerotic lesion development in ApoE-knockout mice. Arterioscler. Thromb. Vasc. Biol. **19:** 2368–2375

78 Chen Z., Fitzgerald R. L., Saffitz J. E., Semenkovich C. F. and Schonfeld G. (2003) Amino terminal 38.9% of apolipoprotein B-100 is sufficient to support cholesterol-rich lipoprotein production and atherosclerosis. Arterioscler. Thromb. Vasc. Biol. **23:** 668–674