HYPOBETALIPOPROTEINEMIA: GENETICS, BIOCHEMISTRY, AND CLINICAL SPECTRUM

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Abbreviations

ABL	abetalipoproteinemia
apoB	apolipoprotein B
CVD	cardiovascular disease
CM	chylomicron
CMRD	chylomicron retention disease
COP	coat protein
CHD	coronary heart disease
ER	endoplasmic reticulum
ERAD	endoplasmic reticulum associated degradation
FHBL	familial hypobetalipoproteinemia
HBL	hypobetalipoproteinemia
LDL-C	LDL-cholesterol
LDL-R	LDL-receptor
MTP	microsomal triglyceride transfer protein
PERPP	post-ER presecretory proteolysis
PCSK9	proprotein convertase subtilisin/kexin type 9
TC	total cholesterol
TG	triglyceride

1. Abstract

Hypobetalipoproteinemias (HBL) represent a heterogeneous group of disorders characterized by reduced plasma levels of total cholesterol (TC), low density lipoprotein-cholesterol (LDL-C) and apolipoprotein B (apoB) below the 5th percentile of the distribution in the population. HBL are defined as primary or secondary according to the underlying causes. Primary monogenic HBL are caused by mutations in several known genes (*APOB*, *PCSK9*, *MTP*, *SARA2*) or mutations in genes not yet identified. Familial hypobetalipoproteinemia (FHBL) is the most frequent monogenic form of HBL with a dominant mode of inheritance. It may be due to loss-of-function mutations in *APOB* or, less frequently, in *PCSK9* genes. The rare recessive forms of primary monogenic HBL are represented by abetalipoproteinemia (ABL) and chylomicron retention disease (CMRD) due to mutations in *MTP* and *SARA2* genes, respectively. The clinical phenotype of heterozygous FHBL is usually mild, being frequently characterized by fatty liver. The clinical phenotype of homozygous FHBL, ABL, and CMRD is usually severe being characterized by intestinal lipid malabsorption and fat-soluble vitamin deficiency. Secondary HBL are due to several nongenetic factors such as diet, drugs, and disease-related conditions. The aim of this review is to discuss the biochemistry, genetics, and clinical spectrum of HBL and to provide a clinical and laboratory diagnostic algorithm.

2. Introduction

HBL include a heterogeneous group of disorders characterized by reduced plasma levels of TC, LDL-C, and apoB below the 5th percentile of the distribution in the population. LDL, previously known as beta-lipoprotein, plays a key role in the transport of hydrophobic neutral lipids (mainly cholesterol) throughout the body. Since apoB is the main structural protein component of LDL, this lipoprotein class belongs to apoB-containing lipoproteins which also include plasma very low density lipoproteins (VLDL) and chylomicrons (CM) [1].

HBL may be caused by mutations in several known genes or mutations in unidentified genes (primary monogenic HBL) and by several nongenetic factors such as diet, drugs, and disease-related conditions (secondary HBL). In primary HBL, the biochemical phenotype segregates as an autosomal dominant or a recessive trait. The diagnosis of dominant HBL is driven by the multigenerational presence of related individuals carrying the biochemical HBL trait (low LDL-C and apoB plasma levels). The diagnosis of recessive HBL is primed by the presence, early in life, of the biochemical HBL trait associated with a generally severe clinical phenotype in offsprings of normolipidemic parents. Dominant HBL includes FHBL (OMIM 107730), recessive HBL includes ABL (OMIM 200100) and CMRD (OMIM 246700)—also called Anderson's disease [1,2].

The spectrum of the clinical features in primary HBL ranges from absence or paucity of symptoms to a very severe syndrome (e.g., Section 7). In general, the genetic or acquired mechanisms that may reduce LDL-C and apoB plasma levels and cause primary or secondary HBL, respectively, must alter the production, assembly, secretion, or catabolism of apoB-containing lipoproteins.

This chapter critically reviews the current knowledge on the pathophysiology, molecular genetics, and clinical features of HBL, and describes a diagnostic approach that may be helpful in a clinical setting.

3. Pathways of apoB-Containing Lipoproteins Production

3.1. Assembly and Secretion of apoB-Containing Lipoproteins

Liver and intestine are the main site of production of apoB-containing lipoproteins. apoB is expressed primarily in liver and intestine and plays a central role in the transport and metabolism of plasma cholesterol and triglycerides (TG) [1]. In human plasma, apoB occurs in two forms: apoB-100 and apoB-48, which are encoded by the same gene (*APOB*) located on

chromosome 2. apoB-100 (the full-length translation product of apoB mRNA) is a large monomeric protein of 4536 amino acids that is synthesized in the liver. apoB-100 is an essential component of liver-derived VLDLs, IDLs, and LDLs, where it serves as ligand for the LDL-receptor (LDL-R). apoB-48, a peptide consisting of the N-terminal 2152 amino acids of apoB-100 (corresponding to 48% of apoB-100), is synthesized by the intestine and is essential for CM production, the lipoproteins that transport dietary lipids. apoB-48 results from a posttranscriptional modification of apoB mRNA (mRNA editing), which converts a glutamine codon at position 2153 into a stop codon [1]. apoB is essential for the production of apoB-containing lipoproteins. Naturally occurring mutations in *APOB* gene that result in structurally abnormal apoBs (e.g., apoB truncated at the C-terminal end) are associated with a reduction of plasma levels of apoB-containing lipoproteins [1–3] (e.g., Section 4.1.1).

The assembly of VLDL in the liver and CM in the intestine occurs cotranslationally (during the synthesis of apoB): while the C-terminal end of apoB is still being synthesized, the N-terminal portion is translocated across the endoplasmic reticulum (ER) and is assembled as small lipoprotein particles for correct targeting to the pathways of lipoprotein secretion. The apoB is targeted to the ER via the signal sequence (spanning amino acids 1–27); its translocation occurs through the translocon, a proteinaceus channel in the ER membrane [4,5].

3.2. Control of the Secretion of apoB-Containing Lipoproteins

In view of the essential role of apoB in the assembly and secretion of apoB-containing lipoproteins, apoB levels are regulated at multiple levels. Several factors influence the translocation process of newly synthesized apoB. Most relevant is the availability of lipids at the site of apoB synthesis in the ER, which appears to dictate the amount of apoB secreted. In addition, the process of translocation is affected by the characteristics of apoB itself, including length, signal peptide polymorphism, and apoB folding to attain lipid-binding capability, which regulate its ability to assemble into lipoproteins [6]. Successful transport and correct conformation of apoB may lead to its final secretion as a lipoprotein constituent. In the case of lipid shortage, nascent apoB translocation into the ER lumen is inefficient and domains of apoB are exposed to the cytosol, where newly synthesized apoB undergoes rapid intracellular degradation (e.g., Section 3.3) [5]. The N-terminal assembly of lipids onto apoB during translocation requires the microsomal triglyceride transfer protein (MTP), an 894 amino acid protein located in the ER lumen that is a component of a protein complex involved in the early stages of apoB lipidation in liver and intestine [7]. MTP has been shown to bind to

the first 1000 amino acids of apoB which form a domain capable of initiating nascent lipoprotein assembly (i.e., capable of recruiting lipids and facilitating the conversion of apoB into a buoyant lipoprotein particle) [8–12]. This ''lipoprotein initiating'' domain of apoB contains the regions spanning amino acid residues 1–264 and 512–721 or 270–570 involved in the binding with MTP [7,13]. The physical interaction between apoB and MTP is important for the initiation of translocation of the nascent apoB chain and for the cotranslational addition of lipids to this chain [4,5,7]. Via these mechanisms, MTP is believed to avoid improper folding and premature degradation of apoB. The crucial role of MTP in the assembly and secretion of apoB-containing lipoproteins is substantiated by the observation that mutations in the *MTP* gene, which abolish MTP activity, are the cause of ABL, a severe recessive disorder in which VLDL and CM are not secreted (e.g., Section 5.1) [7,14]. Pharmacological inhibition of MTP in cultured cells and *in vivo* results in a dose-dependent inhibition of the secretion of apoB [15,16]. Recent evidence suggests that, in addition to MTP, other molecules with lipid transferase activity in the ER, chaperones, and modifying enzymes (such as PDI, BiP, Grp94, chaperon-like lectins, and cyclophilin) in different subcellular compartments may be involved in the lipoprotein formation [17].

The addition of lipids to apoB is believed to occur in two steps. In the first step, a small amount of lipids is added to apoB during its translation and translocation into the ER lumen. This initial lipidation prevents apoB degradation and leads to the formation of a partially lipidated small lipoprotein particle. In the second step, after apoB translation is completed, the bulk of neutral lipids is added to the primordial lipoprotein particle to form a mature particle [18,19]. The maturation of VLDLs and CMs, which starts in the ER, is completed in the Golgi [20]. Mature VLDLs and CMs present in the lumen of the smooth ER are transported from the ER via specialized vesicles to the Golgi apparatus for secretion [21]. The transport through the secretory pathway is mediated by the coat protein (COP) machinery. The COPII complex functions in ER-derived vesicle formation for anterograde transport. One of the subunits of the COPII complex (Sar1-GTPase) has been found to be critical for the vesicular transport of apoB-containing lipoproteins in rat hepatoma cells [22]. COPII associates with apoB-containing lipoprotein particles and forms ER-derived vesicles that initiate their intracellular transport to the Golgi apparatus before release into the circulation [22]. In humans there are two Sar1 proteins, designated Sar1a and Sar1b (encoded by Sar1-ADP-ribosylation GTPase, *SARA1* and *SARA2* genes, respectively), which differ by 20 amino acid residues. The role of Sar1b in vesicular transport of apoB-containing lipoproteins was demonstrated by the observation that mutations in the *SARA2* gene are the cause of CMRD [23]. Whether the Sar1a isoform plays a distinct role in the transport of

apoB-containing lipoproteins (e.g., is specifically required for VLDL secretion by the liver) is an appealing hypothesis that remains to be tested.

3.3. Intracellular Degradation of apoB and Secretion of apoB-Containing Lipoproteins

In contrast to most secreted proteins, the amount of apoB-containing lipoproteins excreted by the cells is mostly regulated by apoB degradation along the ER/Golgi secretory pathway. Targeting of apoB for degradation occurs when: (i) lipid availability is reduced, (ii) MTP activity is low, (iii) apoB is misfolded, or (iv) under cellular stress conditions. Two intracellular apoB degradation mechanisms have been described: (i) ER associated degradation (ERAD), catalyzed by the ubiquitin–proteasome system, occurs cotranslationally via retrotranslocation when misfolded apoB is present in the cell or lipid supply/availability is reduced; (ii) post-ER presecretory proteolysis (PERPP), achieved by autophagy, which delivers oxidized, aggregated apoB and partially assembled apoB lipoproteins to lysosomes for destruction [24,25]. Thus, intracellular degradation of apoB reduces the assembly and secretion of apoB-containing lipoproteins.

Another mechanism involved in the control of the secretion of apoB-containing lipoproteins is the LDL-R-mediated degradation. LDL-R promotes intracellular degradation of apoB-100 resulting in decreased secretion of VLDL by the liver. LDL-R knockout mice secrete apoB at a higher rate than control mice; this increase is prevented by overexpression of the LDL-R [26]. An *in vivo* turnover study in humans demonstrated that complete deficiency of the LDL-R is associated with an increased apoB production rate [27]. In addition, some naturally occurring mutations in LDL-R have been identified which cause retention of both the mutant LDL-R and apoB within the ER [28]. Taken together, these observations suggest that the LDL-R facilitates the retention of apoB in the ER and its presecretory degradation and possibly also the reuptake of newly secreted apoB-containing lipoproteins on the cell surface.

3.4. Clearance of apoB-Containing Lipoproteins via LDL-Receptor

Most of the LDL particles are cleared from plasma by the liver via the LDL-R. LDL-R-mediated uptake and degradation of LDL is an important determinant of the concentration of LDL in the plasma, as clearly demonstrated by some monogenic disorders (autosomal dominant hypercholesterolemias) affecting LDL-R activity/number or the capacity of LDL to bind LDL-R. These disorders may result from: (i) loss-of-function mutations of *LDL-R* gene (reducing the number of LDL-Rs or their function),

(ii) mutations in *APOB* gene (reducing the affinity of apoB for the LDL-R), or (iii) gain-of-function mutations of *PCSK9* gene (resulting in a reduced number of LDL-Rs on the cell surface) [29].

The role of *PCSK9* gene in the regulation of LDL-R number (and the regulation of plasma LDL level) has received great attention in recent years. *PCSK9* gene, located on chromosome 1, encodes a 692 amino acid protein designated proprotein convertase subtilisin/kexin type 9 (PCSK9). This protein is mainly expressed in the liver where it is produced in the ER as a precursor protein which undergoes cotranslational autocatalytic cleavage in the Golgi to create the processed protease that is secreted. Cell culture and animal models have established that the LDL-R is one of the main downstream targets of PCSK9. Secreted mature PCSK9 binds the LDL-Rs on the surface of hepatocytes and promotes their internalization and degradation in a post-ER complex [30,31]. Thus, PCSK9 exerts its effect on the posttranslational regulation of LDL-R by controlling the LDL-R degradation and the number of receptors available on the cell surface. The mechanism by which PCSK9 reduces the number of LDL-R is only partially known. There is evidence that the secreted form of PCSK9 binds directly to the first epidermal growth factor-like repeat of the extracellular domain of the LDL-R. PCSK9 binding to this site is required for LDL-R degradation [32]. The ability of PCSK9 to promote LDL-R degradation, however, is independent of its catalytic activity [33]. PCSK9 would function as a chaperone molecule that prevents LDL-R recycling to the plasma membrane from endosomes and/or targets LDL-R to the lysosome for degradation [33].

4. Dominant Forms of Primary HBL

FHBL is the main form of dominant primary HBL. FHBL is a genetically heterogeneous disorder which may be due to defective secretion or increased catabolism of apoB-containing lipoproteins. The majority of FHBL patients are heterozygotes; homozygous and compound heterozygous FHBL are very rare.

4.1. FHBL Due to Defective Secretion of apoB-Containing Lipoproteins

The main candidate gene in subjects with FHBL with a dominant inheritance is the *APOB* gene. Approximately 50% of FHBL heterozygotes are carriers of pathogenic mutations in the *APOB* gene (*APOB*-related FHBL) [34]. A large number of *APOB* mutations have been reported to be the cause of FHBL and novel mutations are continually being identified in FHBL subjects [2,3,34,35].

4.1.1. APOB *Gene Mutations Producing Truncated APOBs*

Most *APOB* gene mutations cause the formation of premature termination codons in the apoB mRNA. The translation of these mRNAs leads to the formation of truncated apoBs of various size which, to a variable extent, lose the capacity to form plasma lipoproteins in liver and/or intestine and to export lipids from these organs [2,3,34–37]. Truncated apoBs may or may not be detectable in plasma according to their size. Truncated apoBs longer than apoB-29/30 (i.e., with a size corresponding to 29–30% of that of apoB-100, according to a centile nomenclature) are detectable in plasma (by immunoblot with an anti-apoB antibody), as they are secreted into the plasma as constituents of plasma lipoproteins. The detection of a truncated apoB in plasma suggests the presence of a mutation located in a genomic region spanning from exon 26 to exon 29 of *APOB* gene. Truncated apoBs shorter than apoB-29/30, due to mutations located in the first 25 exons of *APOB* gene, are not detectable in plasma, as they are not secreted [3,34,35]. These short truncated apoBs account for 30% of all *APOB* mutations reported so far in FHBL [34]. Heterozygous FHBL subjects carrying truncated apoBs have a reduced production of apoB-containing lipoproteins in liver and, in some cases, in the intestine, which prevents the formation of VLDL and CM, respectively [38,39]. The production rate (in liver and intestine) of truncated apoBs, as compared with the corresponding wild-type forms of apoB (apoB-48 and apoB-100), is greatly reduced for two main reasons: (i) the reduced lipid-binding capacity of structurally abnormal apoBs (notably short truncations) makes them prone to a rapid intracellular degradation, (ii) the presence of premature stop codons in apoB mRNAs due to frameshift or nonsense mutations may induce a rapid mRNA degradation (nonsense-mediated mRNA decay). In addition, FHBL carriers of long apoB truncations, which are secreted into the plasma, may have an increased removal of truncated apoB-containing lipoproteins by the liver (via the LDL-R) [38] or by the kidney via megalin receptor [40].

The plasma levels of LDL-C and apoB in FHBL heterozygotes carrying truncated apoBs are less than 30% of the values found in age- and gender-matched controls [3,34]. This reduction, which is far below the expected 50% values, is due not only to the extremely low levels of the secreted truncated forms of apoB but also to the reduced production rate of apoB-100 encoded by the normal allele which is 70–75% lower than that found in normal subjects [41,42]. The lower production of normal apoB-100 is the result of a reduced synthesis combined with an increased intracellular degradation.

Very few FHBL homozygotes and compound heterozygotes have been reported. In *APOB*-related FHBL homozygotes or compound heterozygotes carrying apoB truncations, LDL-C and apoB are either not detectable

(if both mutant alleles encode truncated apoB shorter than apoB-29/30) or are 10–20% of the control values (if both mutant alleles or at least one mutant allele encode(s) truncated apoBs longer than apoB-29/30) [34,43].

4.1.2. APOB *Gene Mutations Producing Amino Acid Substitutions in apoB*

Few nonconservative amino acid substitutions in apoB have been reported to be the cause of FHBL [44,45]. Two mutations, R463W and L343V, were found to cosegregate with FHBL in two large Libanese kindred. These mutations involve the N-terminal beta-alpha1 domain of apoB which contains sequence elements shown to be important for the proper folding of apoB [13,46], for the physical interaction between apoB and MTP (the chaperone molecule required for apoB lipidation) [8,10] and for lipid recruitment during lipoprotein assembly and secretion [9]. These missense mutations are associated with a decreased secretion of the mutant apoBs that are retained in the ER because of an increased binding to MTP [44,45]. Other carriers of R463W have been recently identified in Italian, Dutch, and Spanish FHBL subjects, suggesting that R463W may be a recurrent mutation in the population [34,37]. Recently, other missense mutations have been reported to be the cause of FHBL. Five missense *APOB* mutations located within the N-terminal 1000 amino acids of apoB, namely A31P, G275S, L324M, G912D, and G945S, were identified in heterozygous carriers of FHBL in the Italian population. Among these mutations, the A31P substitution in apoB completely blocked apoB-48 secretion when expressed in rat hepatoma cells [47]. In contrast to the two missense mutations L343V and R463W, the A31P mutant did not lead to ER retention as the aberrantly folded protein is degraded intracellularly by proteasomes and autophagosome/lysosome pathway [47].

Plasma levels of LDL-C and apoB in heterozygous carriers of the *APOB* missense mutations are comparable with those present in FHBL due to apoB truncations. The two homozygotes for the missense mutation R463W described so far have barely detectable apoB-containing lipoproteins in plasma [44] as observed in patients homozygotes/compound heterozygotes for short truncated apoBs [43,48].

4.2. FHBL Due to Increased Liver Uptake of apoB-Containing Lipoproteins via LDL-Receptor

The large number of FHBL subjects in whom no *APOB* gene mutations were found suggests that other genes are involved in the pathogenesis of FHBL [3]. In the past few years, loss-of-function mutations in *PCSK9* gene emerged as possible cause of FHBL (*PCSK9*-related FHBL).

Studies in mice had shown that the inactivation of *PCSK9* gene [49] was associated with a marked reduction of plasma cholesterol and LDL-C. This effect was due to an increased plasma LDL clearance, secondary to an increased number of LDL-Rs in the liver. This observation suggested that loss-of-function mutations of PCSK9 in humans would increase the receptor-mediated uptake and catabolism of plasma LDL, possibly resulting in reduced plasma level of LDL and a lipid phenotype similar to that found in *APOB*-related FHBL (e.g., Section 4.1). This hypothesis was confirmed when inactivating mutations in human *PCSK9* (resulting in truncated proteins) were discovered in different populations (African-American, African, Caucasian, and Japanese populations) [50–54]. Heterozygous subjects carrying the loss-of-function mutations causing PCSK9 truncations (C679X, Y142X, A68fsL82X, and W428X) were found to have a reduction of plasma LDL-C levels ranging from 30% to 70% [50–54]. One homozygote for C679X and one compound heterozygote (Y142X/ΔR97) were found to have an 80% reduction of LDL-C with respect to control values [51,52,55].

Population studies have demonstrated that some amino acid substitutions of PCSK9 (p.R46L, p.G106R, p.N157K, p.G236S, p.R237W, p.L253F, p. A443T, and p.S462P) are associated with a significant but variable reduction (ranging from 4% to 30%) in plasma levels of LDL-C [56–59]. All these variants were found to be more frequent in hypocholesterolemic subjects; however, only some of them were demonstrated to be loss-of-function mutations *in vitro*. Taken together, these observations strongly suggest that in humans, loss-of-function mutations of *PCSK9* cause an FHBL phenotype by increasing the hepatic uptake of LDL.

In subjects with *PCSK9*-related FHBL, the magnitude of reduction of plasma LDL-C and apoB appears to be less striking than that observed in subjects with *APOB*-related FHBL in whom the reduction usually ranges from 60% to 80% with respect to control values [34].

PCSK9-related FHBL homozygotes or compound heterozygotes have a less severe reduction of LDL-C and apoB with respect to *APOB*-related FHBL homozygotes/compound heterozygotes [43,48,51,52].

Plasma levels of LDL-C and apoB show large interindividual variability in FHBL heterozygotes, regardless of the gene involved (*APOB* or *PCSK9*) [34,50,60] that may be due to environmental factors (notably the diet) or to genetic factors affecting secretion and catabolism of apoB-containing lipoproteins. For example, it was shown that apoE genotype accounts for 15–60% of this variation in *APOB*-related FHBL heterozygotes carrying truncated apoBs [61]. It is also possible that factors affecting intestinal cholesterol absorption (i.e., variations in *NPC1L1* gene) play a role in regulating apoB and LDL-C concentration in plasma of FHBL subjects [62].

4.3. Prevalence of Primary FHBL

Epidemiological data on the prevalence of primary FHBL are scarce. The population frequency of heterozygous *APOB*-related FHBL has been estimated to be 1 in 3000 [63]. Heterozygous *PCSK9*-related FHBL seems to be rare among Caucasians, while in African-Americans this disorder due to the two nonsense mutations (Y142X and C679X) has a prevalence of 2–2.6% [50]. The prevalence of homozygous and compound heterozygous *APOB*- and *PCSK9*-related FHBL is exceedingly rare [2,59].

5. Recessive Forms of Primary HBL

Two very rare primary monogenic HBLs, usually diagnosed in infancy, segregate as autosomal recessive traits: ABL and CMRD. These disorders are characterized by the complete absence of apoB-containing lipoproteins (ABL) or by a selective absence of apoB-48-containing lipoproteins (CMRD) due to a defective assembly and secretion of these lipoproteins by liver and/or intestine [1].

5.1. Abetalipoproteinemia

ABL is an ''exceedingly rare'' disorder that occurs in less than one in 1 million individuals.

The plasma lipid profile of ABL patients is characterized by extremely low plasma levels of TC, VLDL, and LDL and an almost complete absence of apoB-100 and apoB-48. ABL-obligate heterozygotes have normal plasma lipids; in some cases, however, a mild reduction of TC and LDL-C has been reported [14]. ABL is due to mutations in the *MTP* gene which is required for the assembly and secretion of apoB-containing lipoproteins in both liver and intestine (e.g., Section 3.2). A variety of mutations in this gene, located on chromosome 4, have been described; most of them result in truncated proteins devoid of function [14,48,64–68]. Some *MTP* missense mutations have also been reported, which affect either the apoB-binding ability of MTP or its interaction with other components of the protein complex; they are associated with a milder form of the disease [65,66]. It is conceivable that the severity of ABL phenotype is related to the residual activity of MTP and the capacity to form VLDL and CM. The absence of MTP activity leads to the accumulation of large lipid droplets in the cytoplasm in hepatocytes and enterocytes.

5.2. Chylomicron Retention Disease

CMRD is a very rare recessive disorder characterized by the selective absence of apoB-48 in plasma, low plasma cholesterol, and fat-soluble vitamins. apoB-48-containing lipoproteins are not secreted into the plasma,

neither fasting nor postprandially [1]. In CMRD the plasma levels of LDL-C and apoB are 25–40% of the control levels [69].

Affected subjects have an inability to export dietary lipids as CMs, leading to a marked accumulation of CM-like particles in membrane-bound compartments of enterocytes, which contain large cytosolic lipid droplets. CMRD is due to mutations in the *SARA2* gene belonging to the Sar1-ADP-ribosylation factor family of small GTPases. The *SARA2* gene, located on chromosome 5, encodes the Sar1b protein, a single polypeptide of 198 amino acids [23,70] that is involved in the control of the intracellular trafficking of CMs in COPII-coated vesicles. CMs are selectively recruited by the COPII machinery for transport through the cellular secretory pathway [21,22]. Thus, CMRD may arise as a result of defects in the transport of CMs through the secretory pathway.

Up to now, 14 mutations in the *SARA2* gene have been identified in patients with the clinical diagnosis of CMRD-Anderson disease [23,71–74]. Missense mutations of *SARA2* represent the most common cause of CMRD; these mutations were found in the Sar1b β-sheets region and are predicted to perturb the geometry of the guanosine diphosphate and guanosine triphosphate binding site of Sar1b [70]. Only two frameshift mutations have been reported so far [72,74].

6. Primary Orphan FHBL

In a significant number of FHBL kindred, the search for causative mutations in the candidate genes is often inconclusive. Orphan FHBL kindred in which the HBL phenotype segregates as dominant trait have been studied by linkage analysis in order to identify the causative genes. Susceptibility loci have been identified in chromosomes 13q, 3p21.1-22, and 10 but no candidate genes have emerged so far [75–77].

7. Spectrum of Clinical Manifestations in Primary HBL

Monogenic hypobetalipoproteinemias are characterized by a wide spectrum of clinical features.

The clinical phenotypes of primary HBL due to defective secretion of apoB-containing lipoproteins (*APOB*-related FHBL, ABL, and CMRD) are highly variable, their severity being directly related to the degree of the impairment of the production of apoB-100 and/or apoB-48 [3]. The clinical phenotype is more severe in the primary recessive HBLs: in ABL and in CMRD the impaired formation/secretion of apoB-48 explains the severe

intestinal lipid malabsorption and the related disorders (such as the growth retardation and the neurological manifestations caused by vitamin E deficiency) [78,79]. ABL displays a cluster of severe clinical manifestations such as malabsorption of dietary fat and fat-soluble vitamins, steatorrhea, ''failure to thrive'' and acanthocytosis that are present in infancy and childhood. Later in life, because of the deficiency of fat-soluble vitamins, coagulopathy, posterior column neuropathy and myopathy, anemia, spinocerebellar ataxia, and retinitis pigmentosa develop [78]. Few reports have documented a possible association of ABL with ileal adenocarcinoma [80] and metastatic spinal cord glioblastoma [81] and fatty liver was reported in some cases [82,83].

In CMRD, the clinical phenotype is strictly related to the impaired capacity to form CMs after a fat-containing meal. This leads to the development of steatorrhea, growth retardation, malnutrition, and an accumulation of lipid droplets within the enterocyte [84,85]. No neurological symptoms have been reported. However, the description of variable clinical phenotypes and a weak genotype–phenotype correlation in CMRD suggest that this disease might represent a more complex trait rather than a simple autosomal recessive disorder [69,74,79].

FHBL heterozygotes are generally asymptomatic but most of them develop fatty liver and sometimes a mild intestinal malabsorption [34,35]. FHBL homozygotes or compound heterozygotes have fatty liver and may suffer from severe fat malabsorption [43,48].

In homozygotes or compound heterozygotes of *APOB*-related FHBL carrying truncated apoBs, the severity of the clinical phenotype depends on the capacity of the truncated apoBs to bind lipids and to be secreted as lipoprotein particles by liver and intestine. apoBs shorter than apoB-29/30 are degraded intracellularly, mimicking an ABL-like condition [43,48]. The presence in these individuals of at least one mutant *APOB* allele producing truncations longer than apoB-48 may allow the secretion of small amounts of apoB-containing lipoproteins (specifically CMs), which contribute to mitigate the clinical phenotype [34,43]. In homozygotes/compound heterozygotes for long truncations (truncations longer than apoB-48), the clinical phenotype is usually mild, being characterized only by fatty liver [34]. Heterozygous *APOB*-related FHBL carrying short truncations (i.e., truncated apoBs shorter than apoB-48) usually have fatty liver and mild intestinal lipid malabsorption [34,86]; carriers of long truncations are generally asymptomatic but they tend to develop fatty liver [34]. Carriers of apoB missense mutations may be asymptomatic or manifest mild fatty liver disease and sometimes a mild fat malabsorption [44,45,47,87].

A common characteristic of the severe forms of primary HBL is fat-soluble vitamin deficiency, including vitamin E, secondary to fat malabsorption.

Before the introduction of high-dose vitamin (A, D, K, and E) therapy, the majority of ABL patients did not survive past the third decade of life. The long-term vitamin treatment in ABL patients [78] was able to arrest the progression of the neurological complications, especially when initiated before the 16th month of life [88]. The current standard treatment for ABL, which can be extended to CMRD and homozygous FHBL, is based on a low fat diet eventually supplemented with medium-chain triglyceride and the administration of fat-soluble vitamins [79].

The rare cases of *PCSK9*-related FHBL homozygotes, despite a substantial reduction of plasma LDL-C (0.36–0.41 mmol/l), appear to be in good health since they are free of gastrointestinal symptoms and detectable increase in hepatic TG [51,52]. FHBL heterozygotes carrying *PCSK9* mutations are completely asymptomatic in contrast with *APOB*-related FHBL. This difference is probably due to the biochemical pathways underlying *PCSK9*- and *APOB*-related FHBL. Increased hepatic catabolism of LDL present in *PCSK9*-related FHBL does not result in lipid accumulation within the hepatocytes or entherocytes, while such an accumulation is a key feature in the disorders characterized by defective assembly/secretion of apoB-containing lipoproteins (*APOB*-related FHBL, ABL, and CMRD).

8. Main Clinical Issues of FHBL

8.1. FHBL and Fatty Liver Disease

In *APOB*-related FHBL the presence of fatty liver has been documented by abdominal ultrasound examination, magnetic resonance, or liver biopsy [89–93]. In FHBL heterozygous carriers of truncated forms of apoB, the mean liver TG content is approximately fivefold that of controls with a great interindividual variability despite similar apoB truncations and similar measures of obesity and glucose tolerance; this could indicate that other modifier genes than *APOB* may affect the magnitude of hepatic TG accumulation [94]. Histologically fatty liver of FHBL is similar to nonalcoholic fatty liver disease, which is highly prevalent in the general population, often being associated with obesity, dyslipidemia, hypertension, insulin resistance, type 2 diabetes mellitus; all these features, when clustered, characterize the metabolic syndrome [95,96]. In contrast to metabolic syndrome, in FHBL subjects the increased hepatic fat content is neither associated with glucose intolerance nor with insulin resistance [97].

The pathophysiology of fatty liver in *APOB*-related FHBL has been elucidated in animal models: apoB truncation-inducing mutations lead to the production of VLDL particles smaller than normal, with reduced

capacities to bind TG; in addition apoB-100 produced by the normal allele is also reduced. The two combined defects of the VLDL export system produce the accumulation of TG in hepatocytes [98,99].

Fatty liver is also present in FHBL heterozygotes carrying missense mutations of *APOB*; these mutant apoBs are poorly lipidated and secreted as lipoprotein particles, causing an impaired export of hepatic TG [25].

The long-term outcome of fatty liver in FHBL is still unknown; anecdotal reports have documented an association between fatty liver and steatohepatitis, liver cirrhosis and hepatocarcinoma in FHBL patients [86,89–91,100]. Interestingly, fatty liver is not present in other forms of primary HBL such as *PCSK9*-related FHBL [51] and the orphan FHBL (linked to chromosomes 3 and 10 loci) [76,77].

8.2. FHBL and Cardiovascular Disease

Elevated LDL-C is a major risk factor for cardiovascular disease (CVD). A recent meta-analysis of 61 prospective studies demonstrated that higher TC levels were associated with higher risks of coronary heart disease (CHD) and that there is no lower threshold. The association is log-linear suggesting that greater LDL-C absolute reductions should be associated with greater proportional reductions in risk of CHD [101]. In view of the low plasma LDL-C levels, it is reasonable to assume that primary HBL subjects: (i) might be naturally protected against CVD owing to reduced life-time exposure to atherogenic apoB-containing lipoproteins and (ii) represent a unique setting to verify the effects of a lifelong exposure to very low levels of LDL-C on the development of cardiovascular atherosclerosis-related diseases. To date no prospective studies have tested this hypothesis mainly because of the relatively small size of existing FHBL case series. Recently 14 *APOB*-related FHBL subjects have been clinically evaluated and found to be completely free from CVD [37]. A study designed to assess the noninvasive surrogate markers of CVD in FHBL showed that heterozygous *APOB*-related FHBL subjects (no. 41) were found to have a significant decrease in arterial stiffness as compared to control subjects, despite an increased prevalence of traditional cardiovascular risk factors [102].

Convincing evidence of the protection of lifelong reduction of plasma LDL-C has emerged with the observation that individuals with *PCSK9*-related FHBL have a marked reduction of CHD risk. Cohen *et al.* [103] examined the effect of PCSK9 loss-of-function mutations associated with reduced plasma levels of LDL-C on the incidence of CHD in a large population (Atherosclerosis Risk in Communities Study, ARIC). During a 15-year follow-up period among the carriers of the C679X mutation and the R46L variants, there was an 88% and a 47% reduction in the risk of CHD,

respectively. These findings represent a strong ''proof of concept'' in favor of earlier initiation of lifestyle or, when appropriate, of drug interventions to lower LDL-C levels.

8.3. FHBL and Cancer

The association between low plasma cholesterol concentrations and increased risk of cancer has been reported in several studies and it has been explained as an example of ''reverse causality'' (subclinical cancer could reduce cholesterol levels) [104–106]. To test whether long-term low plasma cholesterol levels may increase cancer risk, cancer incidence has been evaluated in the ARIC study, among *PCSK9*-related FHBL [107]. There was no evidence that cholesterol-lowering variants of PCSK9 were associated with increased risk of total cancer.

9. Secondary Hypobetalipoproteinemias

Environmental factors and several diseases may contribute to reduce the LDL-C and apoB plasma levels.

Vegetarians who do not eat meat, fish, diary, or eggs have mean plasma TC and LDL-C levels lower than expected values of the control population. TC and LDL-C average plasma levels are 3.10–3.36 and 1.55–1.81 mmol/l, respectively, which are very close to the 5th percentile of the population distribution [108,109].

In patients with chronic parenchymal liver disease, lipid and lipoprotein plasma levels tend to be reduced [110]. The TC and LDL-C decrease parallels, the impairment of the hepatic synthesis due to the progressive liver failure [111]. Among cirrhotic patients classified according to the MELD score which determines the residual liver function, TC ranges between 3.88 and 2.58 mmol/l and LDL-C between 2.07 and 1.29 mmol/l [112].

Conditions like chronic pancreatitis (adults) and cystic fibrosis (children) are characterized by intestinal fat malabsorption which is associated with HBL. In these common diseases, exocrine pancreatic insufficiency causes steatorrhea when pancreas function is below 5% of normal function. Patients with chronic pancreatitis suffer from malabsorption and nutritional deficiencies. In advanced disease, TC and LDL-C are generally low, 3.10–4.14 and 1.81–2.33 mmol/l, respectively [113]. In cystic fibrosis, the presence of pancreatic insufficiency is associated with lower levels of TC (2.58–2.32 mmol/l) and LDL-C (1.29–1.81 mmol/l) [114].

In end-stage renal disease patients on hemodialysis, the LDL-C distribution curve is shifted to the left [115]; malnutrition and inflammation are

believed responsible for the hypocholesterolemia associated with this condition [116].

Hyperthyroidism exhibits an enhanced excretion of cholesterol, a reduced output of hepatic VLDL and an increased turnover of LDL resulting in a decrease of plasma TC and LDL-C [117,118]. Low plasma levels of TC have been frequently described in a variety of hematologic disorders characterized by the presence of anemia [119]. The existence of HBL has been reported in all phenotypes of beta-thalassemia with TC and LDL-C levels in homo- and heterozygotes about 50% lower compared with healthy controls [120,121]. The mechanisms underlying the hypocholesterolemia observed in beta-thalassemia patients are unknown, and several explanations have been proposed: anemia, liver dysfunction, increased cholesterol consumption by the bone marrow, hyper-stimulation of the LDL-R by inflammatory cytokines, or an overactive reticuloendothelial system [122]. Hypocholesterolemia has also been documented in Sickle cell disease patients and it recognizes the same putative mechanisms as beta-thalassemia [123,124].

Hypocholesterolemia has been associated with cancer; there is no cause–effect relationship but the so-called unsuspected sickness phenomenon [125,126]: subclinical disease may lower cholesterol levels by a LDL-R hyperactivity in tumor cells [127].

10. Conclusions

The plasma levels of LDL-C and apoB below the 5th percentile of the reference population's values represent the biochemical landmark which drives the clinical diagnosis of monogenic hypobetalipoproteinemias. The magnitude of plasma LDL-C and apoB reduction is affected by: (i) the mode of transmission, dominant (*APOB*- or *PCSK9*-related FHBL) versus recessive (ABL and CMRD) and (ii) the heterozygous or homozygous state in the case of FHBL (Table 1).

The first step in the differential diagnosis of HBL is the exclusion of the secondary forms of HBL that are associated with a variety of clinical conditions.

If the clinical diagnosis suggests primary HBL, the study of the transmission of HBL trait in the family of the index case provides a guide for the molecular diagnosis. A mild clinical phenotype (moderate reduction of TC, LDL-C, and apoB possibly associated with fatty liver), when combined with the presence of the HBL trait in family members, strongly suggests the clinical diagnosis of heterozygous FHBL. A diagnosis of possible FHBL should also be considered in a subject with a mild phenotype whenever the mode of transmission of HBL trait is undefined (i.e., it cannot be ascertained

TABLE 1
MONOGENIC PRIMARY HYPOBETALIPOPROTEINEMIAS

Primary HBL	Mode of inheritance	Candidate gene	apoB or LDL plasma levels versus controls	Clinical phenotype
APOB-related FHBL	Codominant	*APOB*	<30%	Heterozygotes: asymptomatic or fatty liver; mild fat malabsorpion in carriers of short truncated apoBs Homozygotes: fatty liver; mild/severe intestinal fat malabsorption (ABL-like phenotype)
PCSK9-related FHBL	Codominant	*PCSK9*	30–70%	Heterozygotes: asymptpmatic Homozygotes: asymptomatic
ABL	Recessive	*MTP*	Undetectable (absence of apoB-containing lipoproteins)	Malabsorption of dietary fat and fat-soluble vitamins, steatorrhea, "failure to thrive," acanthocytosis, neuropathy and myopathy, anemia, spinocerebellar ataxia, retinitis pigmentosa, and fat laden enterocytes (free lipid droplets in the cytoplasm)
CMRD	Recessive	*SARA2*	25–40% (absence of apoB-48-containing lipoproteins)	Steatorrhea, growth retardation, malnutrition, and fat laden enterocytes (free lipid droplets coexist with membrane-bound lipoprotein-like particles)
Orphan FHBL	Codominant	Unknown	<50%	FHBL-like phenotype

ABL, abetalipoproteinemia; APOB, apolipoprotein B; CMRD, chylomicron retention disease; FHBL, familial hypobetalipoproteinemia; MTP, microsomal triglyceride transfer protein; PCSK9, proconvertase subtilisin/kexin type 9.

on firm bases). A severe clinical phenotype (extremely low or undetectable levels of LDL-C and apoB associated with intestinal fat malabsorption and severe fatty liver), notably in children or young adults, suggests the clinical diagnosis of ABL, CMRD, or homozygous FHBL. In case of recessive transmission of the HBL trait, FHBL may be excluded and the differential diagnosis is restricted to ABL and CMRD.

The analysis of candidate genes may reveal the presence of pathogenic mutations (e.g., frameshift, nonsense mutations, or missense mutations known to be pathogenic) or other rare sequence variants with unknown biological effect. In the latter case, the molecular diagnosis remains undefined until cosegregation of the mutant allele with HBL is demonstrated or *in vitro* functional studies of the mutant allele are available.

It should be emphasized that approximately 50% of individuals with the clinical diagnosis of heterozygous FHBL do not have mutations in the two major candidate genes (*APOB* and *PCSK9*) (Orphan FHBL) [34]. This suggests that other major genes affecting the metabolism of apoB-containing lipoproteins remain to be identified.

The flow chart shown in Fig. 1 is provided to guide the clinicians in the identification of the etiology and the molecular bases of HBL.

Addendum

After the submission of the present review, a novel phenotype of primary HBL, designated Familial Combined Hypolipidemia, was reported (Musunuru K et al. N. Engl. J. Med 2010, 363.2220-2227). This disorder, characterized by low plasma levels of LDL-C, TG and HDL-C, was identified in two siblings who were compound heterozygotes for two distinct nonsense mutations in *ANGPTL3* (encoding the angiopoietin-like 3 protein). This finding indicates that loss of function mutations of ANGPTL3 are a novel cause of monogenic HBL.

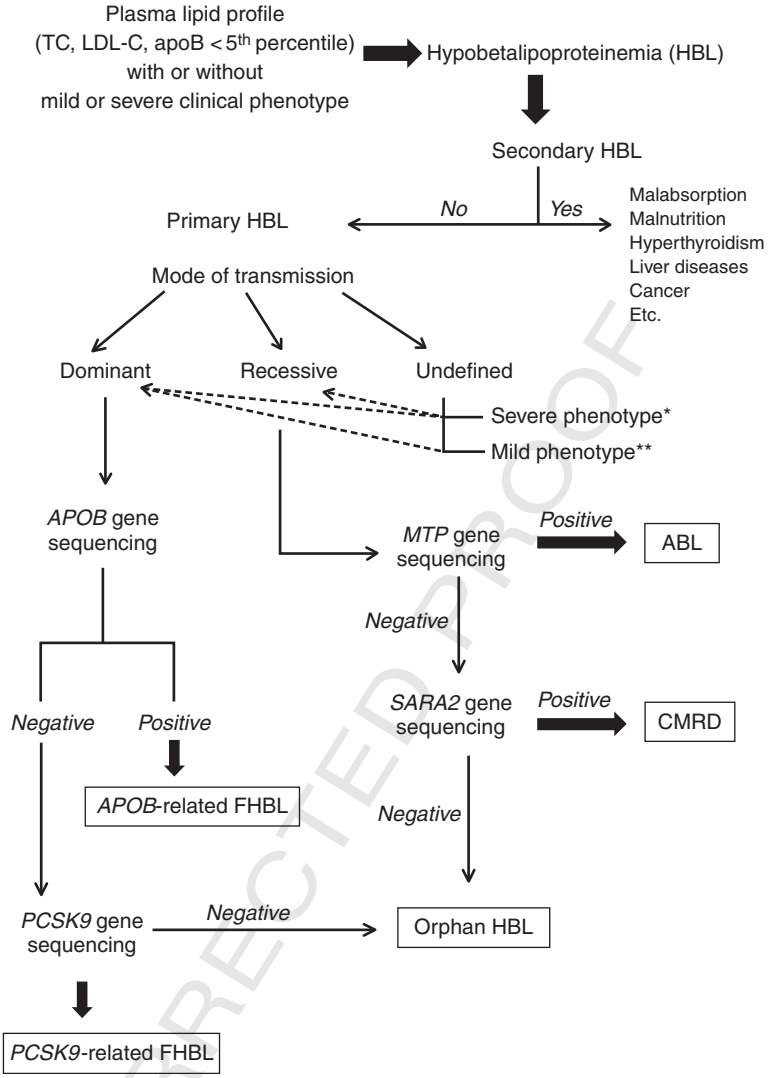

FIG. 1. Flow-chart for the diagnosis of hypobetalipoproteinemias. Mild phenotype** indicates a reduction of plasma TC, LDL-C, and apoB, without relevant clinical manifestations apart from fatty liver. Severe phenotype* indicates a substantial reduction of plasma TC, LDL-C, and apoB associated with clinical manifestations such as intestinal fat malabsorption, growth retardation, fat-soluble vitamins deficiency, severe fatty liver, and possibly neurological disorders. Undefined mode of transmission refers to those HBL cases in which no family history is available. ABL, abetalipoproteinemia; CMRD, chylomicron retention disease; FHBL, familial hypobetalipoproteinemia; HBL, hypobetalipoproteinemia.

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Non-Print Items

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