

# Genetics of familial hypobetalipoproteinemia

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Primary hypobetalipoproteinemias include three monogenic disorders: the relatively frequent codominant familial hypobetalipoproteinemia (FHBL), the rare recessive conditions abetalipoproteinemia (ABL) and chylomicron retention disease (CMRD). Approximately 50% of FHBL patients are carriers of mutations in the *APOB* gene, mostly causing the formation of truncated forms of ApoB. In some kindred, FHBL is linked to a locus on chromosome 3 (3p21), but the candidate gene is still unknown. Recently, a FHBL-like phenotype was observed in carriers of mutations of the proprotein convertase subtilisin/kexin type 9 (*PCSK9*) gene causing loss-of-function of the encoded protein, a proprotein convertase that regulates LDL-receptor number in the liver. Inactivation of the *PCSK9* protein is associated with an increased number of LDL receptors and increased receptor-mediated hepatic uptake of plasma LDL. ABL and CMRD are due to mutations in the microsomal triglyceride transfer protein and Sar1-ADP-ribosylation GTPase 2 genes, which affect assembly and secretion of ApoB-containing lipoproteins. In this review we present the current information on the genetics and pathophysiology of these disorders affecting either the secretion or the catabolism of ApoB-containing lipoproteins.

## Plasma ApoB-containing lipoproteins

Plasma VLDLs, LDLs and chylomicrons (CMs) are designated ApoB-containing lipoproteins, since they contain ApoB as the main structural protein component. ApoB is expressed primarily in liver and intestine and plays a central role in the transport and metabolism of plasma cholesterol and triglycerides [1]. In human plasma, ApoB occurs in two forms: ApoB-100 and ApoB-48, which are encoded by the same gene located on chromosome 2. ApoB-100 (the full-length translation product of ApoB mRNA) is synthesized in the liver. It is one of the largest monomeric proteins known, with 4536 amino acid residues. ApoB-100 is an essential component of liver-derived VLDL and the only apolipoprotein component of LDL, where it serves as a ligand for the LDL receptor (LDL-R). ApoB-48, a peptide consisting of the N-terminal 2152 amino acids of ApoB-100 (corresponding to the 48% of ApoB-100), is synthesized by the intestine and is essential for CM production. ApoB-48 results from a post-transcriptional modification of ApoB mRNA (editing), which converts a glutamine codon at position 2153 into a stop codon [1].

ApoB production is regulated at multiple levels. The transcriptional regulation of *APOB* gene expression in the liver and the intestine is mediated by two sets of very different regulatory elements in separate spatial locations, sharing only the promoter. Regulatory elements extending

upstream and downstream of the ApoB promoter are adequate for hepatic expression of the *APOB* gene, whereas intestinal expression depends on the intestinal control region located upstream of the *APOB* gene [2,3].

Under most physiological conditions, the regulation of ApoB secretion may be governed at the level of translation, translocation and degradation. Structural properties of the 5'- and 3'-untranslated regions of ApoB mRNA, containing sequence elements with the potential to form stable secondary structures, are important regulators of the expression and translation of ApoB mRNA [4]. A newly synthesized ApoB chain must be translocated across the endoplasmic reticulum (ER) membrane for correct targeting to the pathways of lipoprotein assembly and secretion. Successful transport and correct folding of ApoB may lead to its final secretion as a lipoprotein constituent. Several factors influence the translocation process. 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 the case of lipid shortage, newly synthesized ApoB undergoes rapid intracellular degradation, which is accomplished by proteasomal and nonproteasomal pathways [5]. In addition, the process of translocation is affected by the characteristics of ApoB itself, including its conformation, length and signal peptide polymorphism, which regulate its ability to assemble into lipoproteins [2].

**Keywords:**  
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ApoB is essential for the production of ApoB-containing lipoproteins. Naturally occurring mutations of the *APOB* gene in humans causing homozygous familial hypobetalipoproteinemia (FHBL) are associated with an extreme reduction or absence of ApoB-containing lipoproteins [6].

### Assembly & secretion of ApoB-containing lipoproteins

The assembly of VLDL and CM occurs cotranslationally: while the C-terminal end of ApoB is still being synthesized, the N-terminal portion is translocated across the ER and is assembled as small lipoprotein particles. This event not only requires ApoB, but also another protein, designated microsomal triglyceride transfer protein (MTP), an 894 amino acid protein 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 17% of the N-terminal end of ApoB and to be required for the initiation of translocation of the nascent ApoB chain and for the cotranslational addition of lipids to this chain [5,7]. The addition of lipids to ApoB is believed to occur in two steps. In the first step, a small amount of lipid 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 [8,9]. The maturation of VLDL, which starts in the ER, is completed in the Golgi [10].

MTP is crucial for the first step of lipoprotein assembly because it transfers lipids and binds ApoB; via these mechanisms, MTP is believed to avoid improper folding and premature degradation of ApoB. Recent evidence suggests that, in addition to MTP, other molecules with lipid transferase activity in the ER, chaperones and modifying enzymes in different subcellular compartments may be involved in the lipoprotein formation [11].

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 abetalipoproteinemia (ABL), a severe recessive disorder in which VLDL and CM are not secreted (see below) [1,7,12]. Pharmacological inhibition of

MTP in cultured cells and *in vivo* results in a dose-dependent inhibition of the secretion of ApoB [13,14].

Mature VLDL and CM present in the lumen of the smooth ER are transported from the ER via specialized vesicles to the Golgi apparatus for secretion [15]. 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 [16]. 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 [16]. In humans there are two Sar1 proteins, designated Sar1a and -1b (encoded by *Sar1-ADP-ribosylation GTPase [SARA]1* 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 CM retention disease (CMRD) [17]. 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.

Another factor involved in the control of the hepatic secretion of ApoB-containing lipoproteins is the LDL-R. LDL-R-knockout mice secrete ApoB at a higher rate than control mice; this increase is prevented by overexpression of the LDL-R [18]. An *in vivo* turnover study in humans demonstrated that complete deficiency of the LDL-R is associated with an increased ApoB production rate [19].

In addition, some naturally occurring mutations in the LDL-R have been identified that cause retention of both the mutant LDL-R as well as ApoB within the ER [20]. 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 recapture of newly secreted ApoB-containing lipoproteins on the cell surface.

### Primary hypobetalipoproteinemias

The term primary hypobetalipoproteinemia is used to indicate genetic disorders characterized by very low plasma levels of ApoB-containing lipoproteins. Hypobetalipoproteinemia is defined by

plasma levels of LDL-cholesterol (LDL-C) and total ApoB below the 5th percentile of the distribution in the population (<75 mg/dl and 50 mg/dl, respectively) [21].

Primary hypobetalipoproteinemias include three monogenic disorders:

- FHBL, OMIM 107730;
- ABL, OMIM 200100;
- CMRD, OMIM 246700 [1].

### Familial hypobetalipoproteinemia

FHBL is an autosomal codominant disorder with a frequency in the heterozygous form (as estimated by clinical criteria) of 1:500–1:1000 [22].

FHBL heterozygotes are identified either during population screening of individuals with hypocholesterolemia (asymptomatic FHBL) or in the clinical setting during the investigation of subjects with fatty liver and, less frequently, intestinal lipid malabsorption associated with hypobetalipoproteinemia (symptomatic FHBL) [21–24]. The main clinical manifestation of heterozygous FHBL is non-alcoholic fatty liver disease, which defines a wide spectrum of conditions ranging from pure steatosis to nonalcoholic steatohepatitis, in the absence of significant alcohol intake.

Homozygous FHBL is exceedingly rare; in this case the plasma levels of LDL-C and ApoB are extremely low and similar to those observed in ABL (see below). Patients with homozygous FHBL have variable clinical manifestations. Some patients have a severe clinical phenotype (failure to thrive, intestinal fat malabsorption and fatty liver) [1,6,21] while others are asymptomatic [25].

### Molecular genetics of FHBL

FHBL due to mutations in *APOB* gene

FHBL appears to be genetically heterogeneous; it may either be linked or not linked to the *APOB* gene. The best-characterized cases of FHBL are due to mutations of the *APOB* gene. Approximately 80 mutations of this gene have been described, so far, as the cause of FHBL [1,21,23,24]. Most mutations introduce a premature stop codon in ApoB mRNA, which interferes with the complete translation of ApoB mRNA leading to the formation of truncated ApoB proteins of various lengths, ranging from ApoB-2 to ApoB-89 (i.e., from 2 to 89% of the size of ApoB-100, according to a centile nomenclature) [6,21,23,24]. Truncated ApoBs with a size above that of ApoB-29/30 are secreted into the plasma as constituents of plasma lipoproteins; truncated

ApoBs, whose size is below that of ApoB-29/30, are not secreted into plasma either as lipoprotein constituents or in a lipid-free form. Short ApoB truncations, not secreted into the plasma, represent a third of *APOB* mutations reported, so far, in the literature [1,24,26]. In heterozygous FHBL, the production rate (in liver and intestine) of truncated ApoBs is greatly reduced, as compared with the corresponding wild-type forms of ApoB (B-48 and -100), for two main reasons:

- Structurally abnormal ApoBs (notably short truncations) have a reduced capacity to bind lipids, a condition that makes them prone to a rapid intracellular degradation;
- ApoB mRNAs harboring premature stop codons are likely to undergo rapid degradation (nonsense-mediated mRNA decay).

The truncated ApoBs that are secreted into the plasma (ApoBs longer than ApoB-29/30) are usually removed very rapidly from the circulation either by the kidney, via megalin receptors, or by the liver, via LDL-R (if the receptor-binding domain of ApoB is preserved, as in the case of truncated ApoBs longer than ApoB-70) [6,21]. It is possible, however, that lipoproteins containing truncations shorter than ApoB-70 are removed, at least in part, via the LDL-R if they incorporate minute amounts of ApoE, a peptide known to have a high binding affinity for this receptor.

ApoB truncations shorter than ApoB-48 are expected to impair the secretion of ApoB-containing lipoproteins in both liver (VLDL) and intestine (CM); longer truncations only affect hepatic VLDL secretion. This implies that FHBL heterozygotes with truncated ApoBs shorter than ApoB-48 have a reduced capacity of CM production and may manifest intestinal lipid malabsorption, having only one functional *ApoB-48* allele [24,27].

In FHBL heterozygotes with truncated ApoBs, the accumulation of triglycerides in hepatocytes occurs as a result of an impairment of their export via the VLDL system. This is not only due to the reduced production of truncated ApoBs, their impaired capacity to bind lipids and their increased intracellular degradation (see above), but also to a reduced production of the ApoB-100 encoded by the normal allele [6].

The plasma levels of LDL-C and ApoB in FHBL heterozygotes carrying truncated ApoBs are far below the expected 50% values present in normal plasma [21]. This is due to the extremely low levels of truncated ApoBs (the truncated

forms that are secreted) and the reduced production rate of ApoB-100, which is 25–30% of that found in normal subjects (see above).

Only two amino acid substitutions (missense mutations) in ApoB have been reported, so far, to be the cause of FHBL [25,28]. The first missense mutation (arginine to tryptophan conversion at position 463, R463W) involves a domain of ApoB that appears to be critical for the efficient secretion of ApoB and for lipid recruitment during lipoprotein assembly. Expression of recombinant human ApoB-48 carrying the R463W mutation in transfected rat hepatoma cells resulted in markedly decreased secretion efficiency, as compared with wild-type ApoB-48, associated with increased binding to MTP and increased retention of mutant ApoB in the ER [25]. Recently, a second mutation (leucine to valine conversion, L343V), which also impairs the secretion of ApoB-100 and VLDL, has been reported [28]; decreased secretion of the mutant ApoB was associated with increased ER retention and increased binding to MTP. Both R463W and L343V are located within the  $\alpha$ -helical domain in the N-terminus of ApoB, which contains sequence elements shown to be important for the proper folding of ApoB, for the physical interaction between ApoB and MTP and for lipid recruitment during lipoprotein assembly.

FHBL not linked to *APOB* gene

Approximately 50% of FHBL heterozygotes are carriers of pathogenic mutations in the *APOB* gene as revealed by systematic exon-by-exon sequencing of this gene [24]. It is possible that some FHBL patients carry *APOB* mutations that are not detected by routine sequencing (i.e., major rearrangements due to deletion/insertion or mutations in some regulatory sequences in introns or in the upstream 5' flanking region of the *APOB* gene). Regardless of these technical problems, it is a matter of fact that a large proportion of FHBL subjects have no *APOB* gene mutations (*APOB*-negative FHBL) [6], suggesting that other genes are involved. Cosegregation analysis has showed that in some kindred, FHBL does not segregate with the *APOB* locus; in some of them FHBL appears to be linked to a susceptibility locus on chromosome 3p21 [21,29], but the candidate gene is still unknown.

Recent studies have indicated that the pro-protein convertase subtilisin/kexin type 9 (*PCSK9*) gene, located on chromosome 1, is a

new candidate gene in primary hypobetalipoproteinemia. The *PCSK9* protein is mainly expressed in the liver, where it is produced in the ER as a precursor protein. It then undergoes self-cleavage in the Golgi to create the processed protease that is secreted. Secreted *PCSK9* binds the LDL-R on the surface of hepatocytes and promotes their internalization and degradation in a post-ER complex [30,31].

The inactivation of the *PCSK9* gene in mice is associated with a marked reduction of plasma VLDL and LDL [32]. This effect was not due to a defect in the secretion of ApoB-containing lipoproteins by the liver, but as a result of an increased plasma LDL clearance, secondary to an increased number of LDL-R in the liver. It was suggested that *PCSK9*, in some way, exerted its effect on the post-translational regulation of LDL-R by controlling the LDL-R degradation and the number of receptors available on the cell surface. This observation in mice 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 LDL.

In 2005, a population study demonstrated that two inactivating mutations of the *PCSK9* gene (Y142X and C679X producing truncated proteins), found in 2–2.6% of African-American subjects of the Dallas Heart Study, were associated with a 30–40% reduction of plasma LDL-C [33]. Recently, a novel *PCSK9* mutation, resulting in a short truncated protein (L82X), has been reported in Caucasian subjects with the clinical diagnosis of heterozygous FHBL [34]. Other studies have demonstrated that some amino acid substitutions of *PCSK9* (R46L, L253F and A443T) were associated with a significant reduction in plasma levels of LDL-C [35–37]. Finally, a substantial reduction of LDL-C (approximately 80% reduction with respect to control levels) was reported in one homozygous for the C679X mutation [38], in one compound heterozygous carrying the Y142X nonsense mutation and an in-frame deletion resulting in the elimination of an arginine residue at position 97 ( $\Delta$ R97) [39].

Taken together, these observations strongly indicate that *PCSK9* is a plausible candidate gene in FHBL patients negative for *APOB* gene mutations.

The mechanism by which *PCSK9* reduces the number of LDL-Rs 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 [40]. The ability of PCSK9 to promote LDL-R degradation, however, is independent of its catalytic activity [41]. PCSK9 would function as a chaperone molecule that either prevents LDL-R recycling to the plasma membrane from endosomes and/or to target LDL-R to the lysosome for degradation [41].

In conclusion, the reduction of plasma levels of LDL-C and ApoB (the key biochemical trait of FHBL) not only results from defects in hepatic secretion of VLDL – as demonstrated by the mutations in the *APOB* gene affecting the secretion of ApoB-containing lipoproteins – but also from increased tissue uptake and degradation of LDL – as demonstrated by the loss-of-function mutations of the *PCSK9* gene – (Figures 1 & 2).

#### Phenotypic expression of FHBL

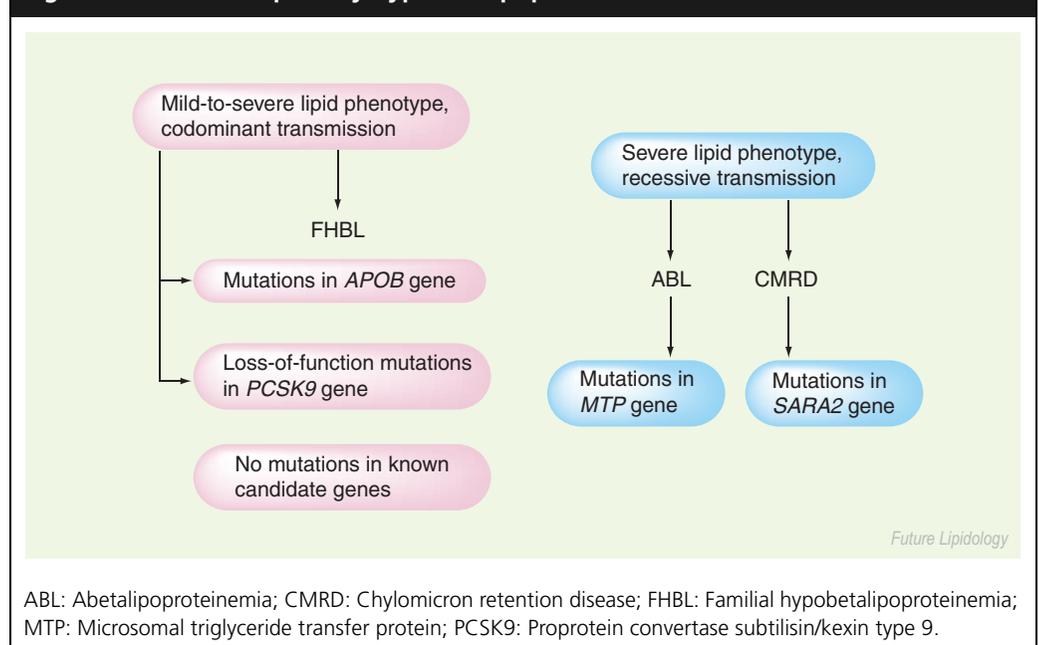
Plasma levels of LDL-C and ApoB show large interindividual variability in FHBL heterozygotes, regardless of the gene involved (*APOB* or *PCSK9*) [24,33,37,42]. This variability may be due to environmental factors (notably the diet) or due to genetic factors affecting secretion and catabolism of ApoB-containing lipoproteins. For example, it was shown that ApoE genotype accounts for 15–60% of this variation in FHBL heterozygotes carrying truncated ApoBs [42]. It

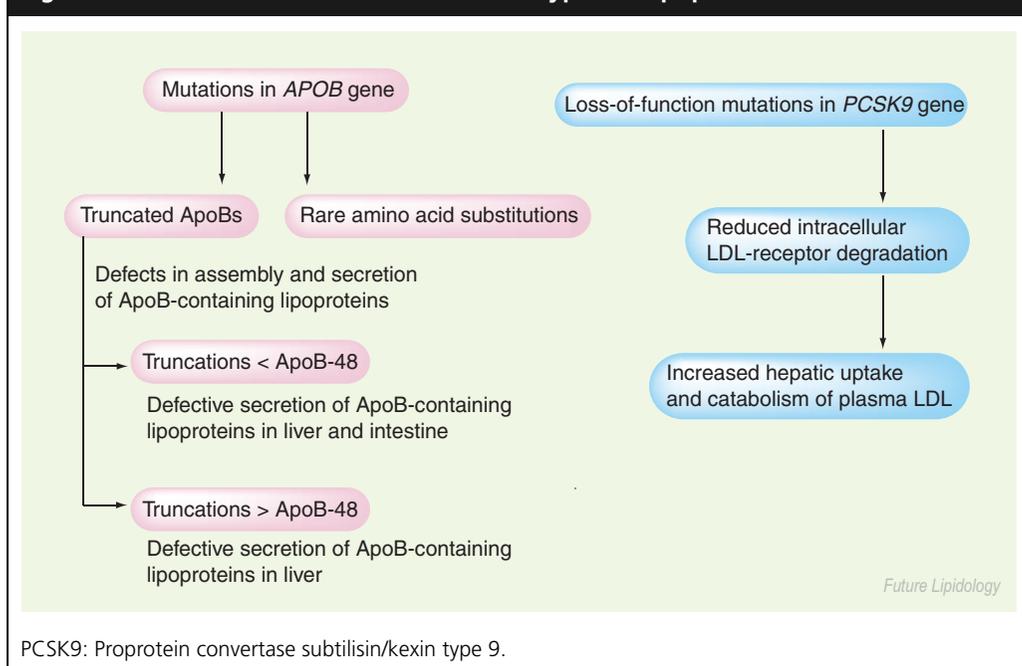
is also possible that factors affecting intestinal cholesterol absorption (i.e., variations in *NPC1L1* gene) play a role in regulating ApoB and LDL-C concentration in plasma of FHBL subjects [43].

One of the key clinical features in FHBL heterozygotes carrying truncated ApoBs is the presence of fatty liver, which is particularly severe in carriers of short truncations [6,24]. Fatty liver has not been reported in FHBL heterozygotes carrying loss-of-function mutations of the *PCSK9* gene [35,39]. This implies that defects in secretion of VLDL play a major role in hepatic triglyceride accumulation.

In view of the low plasma LDL-C levels, subjects with FHBL have been regarded as protected against atherosclerotic coronary heart disease (CHD), owing to reduced life-time exposure to atherogenic ApoB-containing lipoproteins [44]. However, there are no large prospective studies on the prevalence of CHD in FHBL subjects carrying mutations in *APOB* gene. A recent study demonstrated that in these subjects the arterial wall stiffness (taken as an early surrogate marker of preclinical atherosclerosis) was lower than in age- and sex-matched controls [45]. Convincing evidence of the protection of life-long reduction of plasma LDL-C has emerged with the observation that individuals with hypobetalipoproteinemia due to loss-of-function mutations of *PCSK9* have a marked reduction of CHD risk [46].

**Figure 1. Genetics of primary hypobetalipoproteinemias.**



**Figure 2. Molecular mechanisms of familial hypobetalipoproteinemia.**

### Abetalipoproteinemia

ABL is a very rare recessive condition characterized by the absence of ApoB-containing lipoproteins in plasma. Clinical manifestations include failure to thrive, oral fat intolerance, diarrhea, steatorrhea, low plasma level of fat-soluble vitamins, acanthocytosis, atypical retinitis pigmentosa, fatty liver and lipid accumulation in enterocytes [12]. Neurological abnormalities (spinocerebellar ataxia) are a result of deficiencies in fat-soluble vitamins and may manifest later in life, especially if patients have not been given an adequate supplementation of vitamin E [12]. Consanguinity is frequently reported in ABL. The plasma lipid profile of ABL patients is characterized by extremely low plasma levels of total cholesterol, VLDL and LDL and an almost complete absence of ApoB-100 and -48. ABL heterozygotes have normal plasma lipids; in some cases, however, a mild reduction of cholesterol and LDL-C has been reported [47]. All cases of ABL reported, so far, are due to mutations in the *MTP* gene. A variety of mutations in this gene have been described; most of them result in truncated proteins devoid of function [7,12,47–51]. The absence of MTP activity disrupts ApoB-containing lipoprotein assembly in both liver and intestine, leading to the accumulation of large lipid droplets in the cytoplasm. Some MTP missense mutations have also been reported, which affect either the ApoB-binding ability of MTP or its

interaction with the other components of the protein complex; they are associated with a milder form of the disease [49,50].

### Chylomicron retention disease

CMRD (also called Anderson disease) 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, either fasting or post-prandially. 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. Severe fat malabsorption associated with steatorrhea and malnutrition, and failure to thrive in infancy are the main clinical manifestations; no neurological symptoms have been reported [1]. 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 [17] that is involved in the control of the intracellular trafficking of CMs in COPII-coated vesicles [52]. CMs are selectively recruited by the COPII machinery for transport through the cellular secretory pathway. Thus, CMRD may arise as a result of defects in the transport of CMs through the secretory pathways. Recently, eight

different mutations in the *SARA2* gene were identified in patients with the clinical diagnosis of CMRD or Anderson disease [17]. These mutations, resulting in nonconservative amino acid substitutions in the Sar1b protein, represent the most common cause of CMRD. These mutations are located in a region of Sar1b predicted to alter the geometry of GDP, GTP-binding site of Sar1b.

### Conclusion

The understanding of the genetic bases and pathophysiology of primary hypobetalipoproteinemias is important to improve our knowledge on the mechanisms regulating lipid transport and homeostasis of the ApoB-containing lipoproteins. In the future, we hope to extend our knowledge on hypobetalipoproteinemias by identifying new genes and proteins that regulate the intracellular trafficking and secretion of ApoB-containing lipoproteins, as well as their uptake and catabolism in tissues. The discovery of new genes will also give new clues on how sequence variations in specific sets of genes contribute to the variability of plasma levels of LDL and ApoB in the general population, as well as in patients with various forms of primary hypobetalipoproteinemia (notably FHBL). It is reasonable to assume that this body of data will also suggest new therapeutic targets for treating the hyperlipidemias characterized by increased levels of VLDL, LDL and ApoB (such as familial-combined hyperlipidemia), which represent a major risk factor for atherosclerotic cardiovascular disease.

### Future perspective

The study of genetic disorders of ApoB-containing lipoproteins that result in hypobetalipoproteinemia has already provided important insights into some of the key molecular processes regulating whole-body lipid homeostasis. However, several important mechanistic and clinical questions remain. First, although the role of MTP in the assembly and secretion of ApoB-containing lipoproteins is well established, little is known on the:

- Precise mechanisms by which MTP transfers lipids to the nascent ApoB chain
- Role of MTP in the initial lipidation of ApoB and the nucleation of the primordial ApoB-containing particle
- Lipidation of ApoB independent of MTP and factors involved in this process
- Transport of nascent ApoB-containing lipoproteins through the secretory pathway.

The regulation of ApoB production via intracellular ApoB degradation needs to be elucidated. This mechanism is active in hepatic cells, but little is known in enterocytes. In addition, the precise mechanisms (cell condition and signals to target ApoB in the degradation pathway) are not completely clear. The identification of chaperones and protein factors involved in the degradation of ApoB during its transit through the secretory pathway is of utmost interest. In this context, new insights into the role of the COPII machinery in the intracellular transport and secretion of CM and VLDL may lead to the discovery of new pathways and suggest new therapeutic targets to treat some forms of hyperlipidemias, which predispose to atherosclerosis and premature cardiovascular disease.

Second, some issues on FHBL remain to be elucidated. The role of several rare missense mutations in ApoB as the cause of FHBL and their impact on the assembly/secretion of ApoB-containing lipoproteins remains to be established. It is possible that some missense mutations do not affect ApoB secretion, but increase the affinity of ApoB for LDL-R, thus, facilitating LDL uptake by the liver. On the other hand, the search for mutations in the *LDL-R* gene that reduce the affinity of the receptor for PCSK9 resulting in decreased receptor degradation, might represent another mechanism underlying FHBL.

The identification of large families in whom FHBL is not linked to the *APOB* or *PCSK9* genes will represent an important tool for the identification of new genes and new pathways of lipoprotein metabolism. From the clinical standpoint, the main unanswered questions in FHBL concern the following points:

- The genetic and environmental factors affecting fat accumulation in the liver
- The changes of liver fat synthesis and catabolism in the presence of ApoB truncations
- The role of liver fat in promoting degradation of both truncated and wild-type ApoB-100 in hepatocytes
- The potential evolution of fatty liver into more severe chronic liver diseases.

Third, few cases of the rare ABL and CMRD diseases have been reported in the literature. A major international effort should be made to collect data on patients with these disorders, in many countries, in an attempt to define the natural history of these diseases, the results of

treatment (e.g., low-fat diet supplemented with medium-chain fatty acids and fat-soluble vitamin supplementation, etc.) and long-term complications of lipid malabsorption. An area that needs further exploration is the possibility that intestinal cells may have more than one mechanism for the transport of fat and fat-soluble vitamins. The identification of proteins and subcellular transport mechanisms will provide new information about different ways in which the intestine transports fat and fat-soluble vitamins.

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### Executive summary

#### Plasma ApoB-containing lipoproteins

- Study of the pathways of assembly and secretion of ApoB-containing lipoproteins and their regulation in liver and intestine will extend our knowledge on the role of key atherogenic lipoproteins (chylomicrons, VLDL/LDL).

#### Genetics & phenotypic expression of familial hypobetalipoproteinemia

- Familial hypobetalipoproteinemia (FHBL) is genetically heterogeneous and candidate genes may affect several pathways of ApoB metabolism.
- *APOB* gene mutations (mostly truncations) account for approximately 50% of FHBL cases. These truncations prevent or reduce the formation of ApoB-containing lipoproteins in liver alone or in both liver and intestine, depending on their size.
- The proprotein convertase subtilisin/kexin type 9 (*PCSK9*) gene has emerged as a novel candidate gene in FHBL. Loss-of-function mutations of *PCSK9* have been identified as the cause of FHBL; they prevent the intracellular degradation of LDL receptors, thus, increasing the number of surface receptors available for plasma LDL binding, uptake and internalization.
- In some families, FHBL appears to be linked to a locus on chromosome 3p21, but no candidate gene has yet been identified.
- FHBL carrying mutations of *APOB* gene frequently have fatty liver, which might evolve towards more severe liver diseases, especially upon exposure to other liver injuries.

#### Abetalipoproteinemia

- Severe forms of Abetalipoproteinemia (ABL) are usually due to mutations in the microsomal triglyceride transfer protein gene that abolish the function of the encoded protein. Mild forms of ABL caused by mutations with a less severe biological effect may be overlooked or diagnosed only late in life.

#### Chylomicron retention disease

- The genetic defect of chylomicron retention disease has recently been clarified by the discovery of loss-of-function mutations in *SARA2* gene, which encodes a protein (Sar1b) involved in the vesicular transport of chylomicrons in the enterocytes.

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