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GPIHBP1 and the processing of triglyceride-rich lipoproteins

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Summary

GPIHBP1 is a new addition to a group of proteins required for the lipolysis of triglyceride-rich lipoproteins. GPIHBP1 contains an acidic domain and an Ly6 domain with ten cysteines. GPIHBP1 binds lipoprotein lipase (LPL) avidly and likely tethers LPL to the luminal surface of capillaries.

Inactivation of *Gpihbp1* in mice is associated with milky plasma and severe chylomicronemia, even on a low-fat chow diet. Recently, four missense mutations in *GPIHBP1* were identified in humans with severe chylomicronemia (C65Y, C65S, C68G, and Q115P). All four mutations involve highly conserved residues within GPIHBP1's Ly6 domain.

This review will provide an update on GPIHBP1's role in the processing of chylomicrons and the pathogenesis of chylomicronemia.

Keywords

chylomicronemia; lipoprotein lipase; endothelium; mutation; hypertriglyceridemia

Chylomicronemia in *Gpihbp1*-deficient mice

Gpihbp1 was first identified as a chylomicronemia gene in mice [1]. The plasma of homozygous *Gpihbp1* knockout mice is milky, even on a low-fat chow diet, and their plasma triglyceride levels range from 2500–5000 mg/dl. GPIHBP1 is expressed preferentially in tissues that express high levels of LPL—the heart, skeletal muscle, and adipose tissue [1]. In these tissues, GPIHBP1 is located on capillary endothelial cells—the site where LPL-mediated processing of triglyceride-rich lipoproteins is known to occur [1]. Studies with transfected cells have revealed that GPIHBP1 binds LPL quite avidly ($K_d = 3.6 \times 10^{-8}$ M) [1,2]. This constellation of findings led to the idea that GPIHBP1 tethers LPL to the luminal surface of capillaries and serves as a platform for lipolysis. [1].

In addition to serving as a binding site for LPL, GPIHBP1 might also influence the stability of LPL. Angiopoietin-like protein 4 (ANGPTL4) inhibits LPL-mediated processing of lipoproteins by promoting the conversion of active LPL homodimers to inactive monomers [3]. Recent *in vitro* studies suggest that GPIHBP1-bound LPL is protected from ANGPTL4-mediated inactivation [4]. A deficiency of ANGPTL4 ameliorates the hypertriglyceridemia associated with GPIHBP1 deficiency.

The studies with *Gpihbp1* knockout mice revealed four important features of GPIHBP1 deficiency. The first is that GPIHBP1 deficiency is a recessive condition; the plasma lipid

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levels in heterozygous knockout mice (*Gpihbp1*^{+/-}) are entirely normal [1]. Second, the LPL that appears in the plasma of *Gpihbp1*^{-/-} mice after an injection of heparin is enzymatically active [1,5]. Third, after an intravenous injection of heparin, the appearance of LPL in the plasma of *Gpihbp1*^{-/-} mice is delayed [5]. In control mice, LPL is released into the plasma rapidly after an intravenous injection of heparin (within 1 min). In contrast, LPL levels in the plasma of *Gpihbp1*^{-/-} mice increase slowly over 15 minutes after an injection of heparin. Once the heparin-released LPL reaches the plasma of *Gpihbp1*^{-/-} mice, the plasma triglyceride levels fell sharply [5]. Fourth, the stores of LPL in *Gpihbp1*^{-/-} mouse tissues are entirely normal.

These findings implied that the chylomicronemia in *Gpihbp1*-deficient mice is not caused by dysfunctional LPL, but rather by mislocalization of LPL within tissues. We interpreted the heparin infusion studies as providing strong support for this concept [5]. In wild-type mice, we suggested that heparin releases LPL from two different pools—an intravascular pool of LPL, where the LPL is released quickly by heparin, and an extravascular pool, where the LPL is released more slowly. We proposed that *Gpihbp1*^{-/-} mice have only one pool of LPL, the extravascular pool, explaining why LPL entry into the plasma after heparin is retarded (and also explaining why they have chylomicronemia) [5]. Supporting this idea is the observation that an intravenous injection of Intralipid (a triglyceride emulsion, with particles >200 nm in diameter) cannot release any LPL into the plasma of *Gpihbp1*^{-/-} mice, but readily releases LPL into the plasma of wild-type mice [5].

Gpihbp1 knockout mice have provided valuable insights into the atherogenicity of triglyceride-rich lipoproteins [6]. It is commonly assumed, based largely on classic studies from the laboratory of Donald Zilversmit [7], that chylomicrons have limited ability to cause atherosclerosis. However, GPIHBP1 knockout mice developed atherosclerotic lesions. Even though nearly all of the cholesterol and triglycerides in the plasma of *Gpihbp1*^{-/-} mice was associated with large lipoproteins (50–135 nm in diameter), both male and female *Gpihbp1*^{-/-} mice develop atherosclerotic lesions in the aortic root. The lesions were small (median 500 μm^2 /section) at 11–12 months of age but were larger (3000 μm^2 /section) at 16–22 months of age [6]. These lesions are much smaller than those that develop in *Apoe*^{-/-} mice, which have an accumulation of smaller remnant particles. Nevertheless, these studies, along with earlier studies in humans [8] and other mice [9] with chylomicronemia, show that chylomicron-sized particles have some capacity to cause atherosclerosis.

GPIHBP1 and chylomicronemia in humans

A G56R mutation in two siblings with chylomicronemia

Wang and Hegele [10] identified a G56R substitution in a family from Canada (TABLE 1) [10]. Two siblings in the family, both homozygous for the substitution, had severe chylomicronemia. They proposed that the G56R mutation was responsible for the hyperlipidemia because: (1) the family was free of coding sequence mutations in *LPL* or *APOC2*—two other “chylomicronemia genes;” (2) the G56R mutation was a nonconservative substitution in a reasonably well conserved amino acid residue; and (3) they did not find the G56R mutation in 600 normolipidemic control subjects. Later, however, serious doubts were raised about the functional relevance of the G56R substitution, mainly because this substitution had no impact on the ability of LPL to bind LPL (FIGURE 1) [11]. Also, it was noteworthy that three heterozygotes in the G56R kindred had mild hypertriglyceridemia [10]. More recent studies have shown that heterozygotes for a functional defect in GPIHBP1 are normolipidemic [12,13], as is the case for heterozygous knockout mice [1].

Chylomicronemia in a young man who was homozygous for a Q115P mutation in *GPIHBP1*

The Q115P mutation (TABLE 1) was identified in a 33-year-old man with severe chylomicronemia who had no mutations in *LPL*, *APOC2*, or *APOA5* [14]. The patient had had hepatosplenomegaly, lipemia retinalis, but no history of eruptive xanthomas or pancreatitis. A low-fat diet helped control the hyperlipidemia, bringing the fasting plasma triglyceride levels from 3366 to 744 mg/dl. There was no information on the proband's family because he was born in Columbia and subsequently adopted by a Dutch family [14].

Most of the triglycerides in the proband's plasma were associated with large lipoproteins, and the average size of the triglyceride-rich lipoprotein particles was significantly larger than those of a normolipidemic control subject [14]. Plasma apo-B48, apo-CII, and apo-CIII levels were all elevated. The plasma LPL mass levels (from a plasma sample drawn 18 min after an injection of heparin) were low, approximately 10% of levels found in normal subjects, but the specific activity of the proband's LPL seemed to be fairly normal [14].

Cell culture studies revealed that the Q115P mutation reached the cell surface quite normally, but had little, if any, ability to bind LPL. In fact, cells expressing GPIHBP1-Q115P bound <5% as much LPL as cells expressing wild-type GPIHBP1 (FIGURE 1) [14]. These *in vitro* studies strongly supported the idea that the Q115P mutation caused the patient's hyperlipidemia.

Chylomicronemia in a young boy homozygous for a C65Y mutation in *GPIHBP1*

A 3-year-old boy with severe chylomicronemia exhibited failure to thrive, and the body weight was less than the 10th percentile for his age [12]. The boy had lipemia retinalis, and a history of pancreatitis. No hepatosplenomegaly or eruptive xanthoma was detected. The proband's hyperlipidemia was responsive to a low-fat diet, reducing the plasma triglyceride levels from 4005 to 1575 mg/dl [12]. The proband had no mutations in the coding sequences of *LPL*, *LMF1*, *APOC2*, or *APOA5*, but was homozygous for a C65Y mutation in *GPIHBP1* (TABLE 1). The boy's parents (who were first cousins) as well as his three siblings were heterozygous for the C65Y mutation; all were normolipemic [12].

The vast majority of the triglycerides in the proband's plasma were associated with large lipoproteins, as judged by FPLC fractionation studies [12]. Plasma levels of apo-B48, apo-B, apo-CII, and apo-CIII levels were elevated.

The GPIHBP1-C65Y mutant was expressed normally at the surface of transfected cells, but the mutant bound LPL very poorly, if at all, in a cell-based LPL binding assay (FIGURE 1) [12]. These data strongly suggested that the C65Y mutation was responsible for the patient's chylomicronemia.

Chylomicronemia in compound heterozygotes for a C65S and a C68G mutation in *GPIHBP1*

Three of four siblings in a Swedish family had severe chylomicronemia [1]. There was no apparent consanguinity in the family [13]. Two of the affected siblings were females; both had hepatosplenomegaly, bouts of pancreatitis, and more severe hypertriglyceridemia during pregnancies. None of the affected siblings had a history of eruptive xanthomas.

A heterozygous N291S mutation in *LPL* was initially identified in one of the affected siblings, but this mutation was dismissed as a potential cause for the hyperlipidemia when this mutation was found in normolipidemic members of the family [13]. All affected siblings had substantial amounts of apo-CII in their plasma, excluding *APOCII* deficiency as a cause of the hyperlipidemia. Next, the affected siblings were evaluated for mutations in *GPIHBP1*. All three were compound heterozygotes for C65S and C68G mutations in *GPIHBP1* (TABLE 1)

[13]. Both parents were normolipidemic; the father was heterozygous for the C65S mutation, while the mother was heterozygous for the C68G mutation.

Biochemical studies of the GPIHBP1-C65S and GPIHBP1-C68G mutants revealed that both reached the cell surface normally [13]. Neither of these two mutants, however, was able to bind LPL, as judged by both a cell-based binding assay and a cell-free assay [13]. The studies showing defective LPL binding, along with the fact that these mutations were not found in normolipemic control subjects, strongly suggested that the C65S and C68G mutations accounted for the chylomicronemia in the three siblings.

Abnormal LPL levels in the plasma of humans with *GPIHBP1* mutations

LPL mass in pre- and post-heparin plasma—Olivecrona and co-workers [13] showed that the levels of LPL in the pre-heparin plasma of the three C65S/C68G compound heterozygotes were ~5–15% of those in their normolipidemic parents and other normolipemic control subjects. After an intravenous injection of heparin, LPL mass in the plasma of healthy heterozygote relatives [13] or control subjects [12] increased tenfold within 10 min, then reached a plateau for 10 min [12,13], and then gradually returned to pre-heparin levels 60 min after the injection [13]. However, in the case of the C65S/C68G compound heterozygotes [13], as well as the Q115P [12] and C65Y [12] homozygotes, LPL mass after heparin increased slowly for 20 to 60 min, and the absolute levels remained extremely low [12,13]. Indeed, 60 min after the heparin injection, LPL mass levels in the C65S/C68G compound heterozygotes were only ~5% of those in their normolipidemic parents [13].

Plasma lipase activities—The reduced levels of LPL mass in pre- and post-heparin plasma in patients with *GPIHBP1* mutations were associated with a proportional reduction in LPL activity levels. LPL activity in pre-heparin plasma of the C65S/C68G compound heterozygotes was ~10% of the activity found in the plasma of their parents [13]. Even though LPL activity increased in the plasma of the C65S/C68G compound heterozygotes after an injection of heparin, the absolute LPL activity levels were lower than in their parents (6.3, 11, and 7.0 mU/ml in the siblings with chylomicronemia vs. 261 and 130 mU/ml in the normolipidemic parents) [13].

When the Q115P homozygote was infused with heparin for 6 h, LPL was released slowly into the plasma, and the absolute LPL mass levels were far lower than those in normal controls [12]. However, the LPL that did find its way into the plasma of the Q115P homozygote was enzymatically active, and the plasma triglyceride levels fell from 1780 to 534 mg/dl [12].

LPL activity levels as well as LPL synthetic rates were assessed in biopsies of adipose tissue from two of the C65S/C68G compound heterozygotes and healthy control subjects. Interestingly, no differences were observed [13].

Breast milk was obtained from a female C65S/C68G compound heterozygote after she had given birth. Interestingly, LPL mass and activity levels in the breast milk were 2-to 8-fold higher than in milk obtained from control subjects [13]. However, the *lipid content* of milk from the C65S/C68G compound heterozygote was abnormally low. Also, there was a shift towards medium-chain and saturated fatty acids, suggesting that much of the fat in the milk was produced by *de novo* lipogenesis, rather than from lipolysis of triglyceride-rich lipoproteins in the plasma [13].

These data, when considered together, suggest that chylomicronemia patients with defects in *GPIHBP1* have plenty of enzymatically active LPL in their tissues, but that it is mislocalized away from the luminal surface of capillaries and therefore not capable of hydrolyzing

triglycerides in the plasma. GPIHBP1 defects obviously do not affect the entry of LPL into breast milk.

Studies of hepatic lipase (HL) release after an injection of heparin have revealed that pre- and post-heparin HL mass and activity levels are normal in *GPIHBP1*-deficient patients [12,13]. Similar findings were observed in *Gpihbp1*-deficient mice [1].

Recent studies on GPIHBP1 functional domains

Nascent human GPIHBP1 (ENSP00000329266) is a 184-amino acid protein containing a signal peptide as well as a carboxyl-terminal hydrophobic domain, which is ultimately replaced by a glycosylphosphatidylinositol anchor in the mature protein. GPIHBP1 is N-glycosylated, and this glycosylation is required for efficient trafficking of GPIHBP1 to the cell surface [15]. Mature GPIHBP1 contains two noteworthy structural domains—an acidic domain enriched in glutamic and aspartic acid residues (amino acids 25–50) and a cysteine-rich Ly6 domain (spanning from C65 to C136).

The acidic domain is located at the amino terminus of the protein. In human GPIHBP1, 21 out of the 26 amino acids of the acidic domain are either aspartate or glutamate. Lying in the middle of the acidic domain is a conserved tyrosine (Y38 in the human sequence). This tyrosine is predicted to be sulfated, a modification that would render this domain even more negatively charged. Mutation of GPIHBP1's acidic domain abolishes its ability to bind LPL [16]. A complete deletion of the acidic domain also reduces the levels of GPIHBP1 in the cells, suggesting that the acidic domain may be important for the trafficking of GPIHBP1 to the cell surface or the stability of the protein [16].

GPIHBP1's Ly6 domain contains 10 cysteines in a highly characteristic spacing pattern. GPIHBP1 is just one member of a large family of proteins containing cysteine-rich Ly6 domains. The structures of several Ly6 family members, notably UPAR and CD59, have been solved, revealing that each of the 10 cysteines is engaged in a disulfide bond (the first cysteine forms a disulfide bond with the fifth cysteine, the second with the third, the fourth with the sixth, the seventh with the eighth, and the ninth with the tenth) [17,18]. This disulfide bonding pattern results in a characteristic three-fingered structural motif. The structure of GPIHBP1 has not yet been solved, but since the same Ly6 disulfide bonding pattern has been observed in several Ly6 family members [19,20], GPIHBP1's Ly6 domain probably has the same pattern of disulfide bonding.

Recently, Beigneux *et al.* [21] individually changed each cysteine of the Ly6 domain to alanine and then tested the ability of the mutant GPIHBP1 proteins to bind LPL. Before testing LPL binding activity, Beigneux *et al.* [21] were concerned that the cysteine-to-alanine mutations would interfere with trafficking of the protein to the cell surface. However, this was not the case, at least with transiently transfected cells.

Significant amounts of each of the cysteine-to-alanine mutants were detected at the surface of cells, both by immunohistochemistry and western blot analysis [21]. However, none of the mutant GPIHBP1 proteins retained the ability to bind LPL in the cell-based assay (FIGURE 2) or in the cell-free assay [21], suggesting that intact disulfide bonds are required for GPIHBP1 function. In the same study, Beigneux *et al.* [21] showed that the cysteine-to-alanine substitutions did not appear to affect the accessibility of the acidic domain. That result strongly implied that the Ly6 domain plays a direct role in binding LPL. Consistent with that concept, the four GPIHBP1 mutations causing chylomicronemia (C65Y, C65S, C68G, and Q115P) involve highly conserved residues within the Ly6 domain [12–14].

Conclusions

GPIHBP1 plays a critical role in lipolysis of triglyceride-rich lipoproteins. Initially discovered with the aid of *Gpihbp1* knockout mice, this finding has now been amply supported by genetic studies in humans. In both mice and humans, defects in GPIHBP1 cause severe chylomicronemia and are associated with delayed entry of LPL into the plasma after an injection of heparin.

GPIHBP1, a small glycoprotein of the Ly6 family, binds LPL avidly. It is expressed exclusively on microvascular endothelial cells of the heart, skeletal muscle, and adipose tissue, the sites where lipolysis is known to occur. GPIHBP1 contains two main functional domains—an amino-terminus acidic domain and a Ly6 domain. Both are required for LPL binding.

Future perspectives

GPIHBP1 deficiency, both in mice and in humans, is associated with low LPL levels in the pre-heparin plasma and is characterized by delayed release of LPL into the plasma after an injection of heparin. These findings suggest that, in the absence of GPIHBP1, little LPL exists in the lumen of capillaries—the site where LPL hydrolyzes lipoprotein triglycerides. It seems quite possible that GPIHBP1 is required for the transport of LPL into the lumen of capillaries, and when GPIHBP1 is absent, the LPL remains mislocalized to the subendothelial compartment. The mechanism by which LPL finds its way from where it is synthesized (in myocytes and adipocytes) to the lumen of capillaries has been a mystery for more than 50 years. Thus, if GPIHBP1 proves to be the “LPL transporter,” it would solve a long-standing mystery in plasma lipid metabolism.

Another issue worth investigating is the possibility that alterations in GPIHBP1 expression levels or GPIHBP1 function might lead to some cases of acquired chylomicronemia, such as that occurring in some patients with type 2 diabetes.

Finally, it will be important to define the precise amino acid residues in GPIHBP1's Ly6 domain that are important for the binding of LPL. Conversely, it will be important to define the LPL residues involved in the binding to GPIHBP1, and determine whether mutations in those residues would interfere with LPL's capacity to bind to GPIHBP1.

Executive summary

Chylomicronemia in *Gpihbp1*-deficient mice

- *Gpihbp1* knockout mice have severe chylomicronemia, with plasma triglyceride levels of 2500–5000 mg/dl.
- The entry of LPL into the plasma after an injection of heparin is delayed in *Gpihbp1*^{−/−} mice.
- Despite having very large lipoproteins in their plasma, *Gpihbp1*^{−/−} mice are susceptible to atherosclerosis.
- Heterozygous knockout mice (*Gpihbp1*^{+/-}) are normolipidemic.

GPIHBP1 and hypertriglyceridemia in humans

- Four missense mutations (C65Y, C65S, C68G, and Q115P) in the Ly6 domain of GPIHBP1 have been linked to severe chylomicronemia in humans.
- The chylomicronemia associated with the human GPIHBP1 defects is a recessive disorder; heterozygotes are normolipidemic.

- The chylomicronemia associated with GPIHBP1 is ameliorated by a low-fat diet.

Abnormal LPL levels in *GPIHBP1*-deficient humans

- GPIHBP1 deficiency in humans is associated with low LPL levels in both the pre- and post-heparin plasma.
- GPIHBP1 deficiency does not seem to have an impact on LPL synthesis, its enzymatic activity, or the tissue stores of LPL.

Studies on GPIHBP1 functional domains

- GPIHBP1 contains two main structural motifs—an acidic domain and a cysteine-rich Ly6 domain.
- Both the acidic domain and the Ly6 domain are required for binding LPL.

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Abbreviations

GPIHBP1	glycosylphosphatidylinositol-anchored high density lipoprotein-binding protein 1
GPI	glycosylphosphatidylinositol
LPL	lipoprotein lipase
CHO	Chinese hamster ovary
Ly6	Lymphocyte Antigen 6

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Fig. 1. LPL binding properties of mutant GPIHBP1 proteins found in patients with chylomicronemia

Chinese hamster ovary (CHO) cells were electroporated with S-protein-tagged wild-type GPIHBP1, GPIHBP1-C65Y, GPIHBP1-Q115P, GPIHBP1-G56R, or empty vector. The next day, the cells were incubated for 2 h at 4°C with human V5-tagged LPL $\pm$ 500 U/ml of heparin. The cells were washed extensively before preparing cell extracts to assess the amount of bound LPL by western blotting with an anti-V5 antibody. The levels of GPIHBP1 expression were assessed with an antibody against the S-protein tag, and actin was used as a loading control. Reproduced, with permission, from Franssen *et al.* [10].

Fig. 2. The substitution of any cysteine residue in GPIHBP1's Ly6 domain with an alanine abolishes GPIHBP1's ability to bind LPL

CHO cells were electroporated with empty vector, S-protein-tagged wild-type GPIHBP1, or cysteine-to-alanine GPIHBP1 mutants. 24 h later, the cells were incubated with V5-tagged human LPL $\pm$ heparin (500 U/ml). The cells were washed six times, and cell extracts were prepared to assess the amount of LPL bound to the cells by western blotting with a V5-tag-specific antibody. Also, the level of GPIHBP1 in the cell extracts was assessed by western blotting with an anti-S-protein tag antibody. Actin was used as a loading control. This figure was originally published in *J. Lipid Res.* 2009; 284:30240-30247. © the American Society for Biochemistry and Molecular Biology.

Table 1*GPIHBP1* Mutations

Mutation	Consequences for GPIHBP1	Clinical observations	Refs.
c.166G>C	G56R substitution No effect of this mutation on the ability of GPIHBP1 to bind LPL	Identified in two siblings with chylomicronemia; relapsing pancreatitis; coronary artery disease	[10]
c.194G>C	C65S substitution This mutation abolishes the ability of GPIHBP1 to bind LPL.	Fasting chylomicronemia, partially responsive to diet; mild pancreatitis, hepatosplenomegaly	[13]
c.194G>A	C65Y substitution This mutation abolishes the ability of GPIHBP1 to bind LPL.	Fasting chylomicronemia, partially responsive to diet; pancreatitis, lipemia retinalis; no hepatosplenomegaly or eruptive xanthoma were noted.	[12]
c.202T>G	C68G substitution This mutation abolishes the ability of GPIHBP1 to bind LPL.	Fasting chylomicronemia, partially responsive to diet; mild pancreatitis, hepatosplenomegaly	[13]
c.344A>C	Q115P substitution This mutation abolishes the ability of GPIHBP1 to bind LPL.	Chylomicronemia, partially responsive to diet; hepatosplenomegaly; lipemia retinalis; no history of pancreatitis or eruptive xanthoma	[14]

Amino-acid sequence of human GPIHBP1¹:

1 *MKALGAVLLALLLCGRPGRGQTQEEEEDEDHGPDDYDEEDEDEVEEEETNRLPGGRSRVLLRCYT*
68 *CKSLPRDERCNLTQNC SHGQTCTTLIAHGNTESGLLTTHSTWCTDSCQPITKTVEGTQVTMTCCQSS*
135 *LCNVPPWQSSRVQDPTGKGAGGPRGSSETVGAALLNLLAGLGAMGARRP*

¹The position of the GPIHBP1 missense mutations in patients with severe hypertriglyceridemia are noted by bold font; the signal peptide is italicized; the Ly6 domain (thick line) is underlined; the acidic domain is underlined with a dashed line.