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GPIHBP1—an endothelial cell molecule important for the lipolytic processing of chylomicrons

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Abstract

Purpose of review—This review summarizes recent data indicating that glycosylphosphatidylinositol-anchored high density lipoprotein-binding protein 1 (GPIHBP1) plays a key role in the lipolytic processing of chylomicrons.

Recent findings—Lipoprotein lipase (LpL) hydrolyzes triglycerides in chylomicrons at the luminal surface of the capillaries in heart, adipose tissue, and skeletal muscle. However, the endothelial cell molecule that facilitates the lipolytic processing of chylomicrons has never been clearly defined. Mice lacking GPIHBP1 manifest chylomicronemia, with plasma triglyceride levels as high as 5,000 mg/dl. In wild-type mice, GPIHBP1 is expressed on the luminal surface of capillaries in heart, adipose tissue, and skeletal muscle. Cells transfected with GPIHBP1 bind both chylomicrons and LpL avidly.

Summary—The chylomicronemia in *Gpihbp1*-deficient mice, the fact that GPIHBP1 is located within the lumen of capillaries, and the fact that GPIHBP1 binds LpL and chylomicrons suggest that GPIHBP1 is a key platform for the lipolytic processing of triglyceride-rich lipoproteins.

Keywords

Chylomicronemia; lipoprotein lipase; hypertriglyceridemia; GPI-anchored proteins

Introduction

The triglycerides in chylomicrons are hydrolyzed by lipoprotein lipase (LpL) along the luminal surface of the capillaries. Substantial evidence has pointed to a role for heparan sulfate proteoglycans (HSPGs) in anchoring both LpL and chylomicrons to the surface of capillaries. However, recent data has highlighted the importance of a new endothelial cell molecule—glycosylphosphatidylinositol-anchored high density lipoprotein-binding protein 1 (GPIHBP1)—in the lipolytic processing of chylomicrons. In this review, we summarize data suggesting that GPIHBP1 is an important platform for the lipolytic processing of chylomicrons.

Metabolism of Triglyceride-Rich Lipoproteins in the Plasma

Dietary fats are packaged into chylomicrons in the intestine [1]. Chylomicrons are initially secreted into the lymphatics but reach the systemic circulation *via* the thoracic duct. In the systemic circulation, chylomicrons acquire apo-E as well as more of the C apolipoproteins

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from HDL. The acquisition of apo-CII is important because it is a cofactor for LpL [1–5]. The acquisition of apo-E may also assist in lipolysis, as the positively charged heparin-binding domain of apo-E could bind to negatively charged HSPGs lining the capillary wall, thereby facilitating lipolysis [6,7]. Adding extra apo-E to triglyceride-rich lipoproteins increases their ability to bind to the surface of isolated blood vessels *in vitro* [8].

Triglycerides within the core of chylomicrons are hydrolyzed by LpL, mainly in adipose tissue, heart, and skeletal muscle [7]. LpL requires a protein cofactor, apo-CII, for activity [9]. Most investigators have assumed that LpL binds to HSPGs on the capillary surface through the interaction of the heparin-binding domains in LpL with negatively charged HSPGs [10]. It is not entirely clear how the triglyceride-rich lipoproteins are delivered to the active site of LpL. However, LpL clearly binds to lipoproteins, and many have assumed that lipoproteins bind directly to LpL within capillaries [11].

Chylomicrons are rapidly metabolized; their residence time in the circulation is only of a few minutes [1,5]. The hydrolysis of lipoprotein triglycerides by LpL provides fatty acids for fuel in muscle and heart or for storage as triglycerides in adipose tissue [7]. Also, the surface components of triglyceride-rich lipoproteins contribute to the biogenesis of HDL, and the lipolytic processing of hepatic VLDL leads to the formation of LDL, an atherogenic lipoprotein [12]. Thus, lipolysis occupies a central position in lipid and lipoprotein metabolism [12,13].

Lipolysis of Triglyceride-rich Lipoproteins by LpL

LpL is a 448-amino acid glycoprotein that is active as a dimer. LpL is organized into two structurally distinct regions—an amino-terminal domain and a smaller carboxyl-terminal domain—which are connected by a flexible peptide. The amino-terminal domain contains the catalytic triad (Ser-132, Asp-156, and His-241) responsible for lipolysis [14]. The carboxyl-terminal domain contains the dominant heparin-binding domain and is thought to be important for binding lipoproteins [15–17]. LpL contains five disulfide bridges that are critical for LpL dimerization and dimer stability [16].

LpL binds to HSPGs on cells, and specifically to the glycosaminoglycan (GAG) chains of proteoglycans. There is no evidence that LpL binds to the core proteins of HSPGs. In cultured adipocytes, removal of cell-surface heparan sulfate chains with heparinase or heparitinase [18] significantly increases “LpL secretion” into the medium, and little LpL remains associated with the cell surface. Similar results are observed in cells treated with sodium chlorate, which reduces the sulfation of proteoglycans [19]. These observations are not inconsistent with the idea that another molecule such as GPIHBP1 acts as a key binding site for LpL. It is possible that LpL binds to HSPGs and that some of this pool of LpL is transferred to GPIHBP1. There is a precedent for this type of scenario: HSPGs bind fibroblast growth factors, even though those molecules also have dedicated high-affinity receptors [20].

Apo-AV has emerged as a regulator of the lipolysis of triglyceride-rich lipoproteins [21,22]. Plasma triglyceride levels are increased fourfold in *Apoav*-deficient mice and reduced by 70% in transgenic mice that overexpress apo-AV [21]. Also, various polymorphisms in apo-AV have potent effects on plasma triglyceride levels in human population studies [21]. An apo-AV truncation mutation in humans is associated with severe hypertriglyceridemia [23]. Recent studies have indicated that apo-AV improves the efficiency of lipolysis. The proposed model [24] is that apo-AV increases the binding of triglyceride-rich lipoproteins to HSPGs on the surface of the capillary endothelium, thereby facilitating lipolytic processing by LpL. The plasma concentrations of apo-AV are remarkably low [24–26], probably only 0.1% of the concentration of apo-AI, but apo-AV clearly has a large impact on plasma triglyceride metabolism.

LpL is highly expressed in heart, adipose tissue, lactating mammary gland, and skeletal muscle [27]. In the fed state, LpL is upregulated in white adipose tissue and downregulated in skeletal muscle and heart. During fasting, this pattern is reversed. Thus, in a setting of energy restriction, triglyceride-rich lipoproteins are hydrolyzed in tissues that require oxidative fuel and not by adipose tissue [27]. LpL expression in specific tissues is regulated by both transcriptional and posttranscriptional mechanisms [27]. The half-life of *LPL* mRNA, approximately 17 h, is too long to explain the rapid changes in enzyme activity in response to fasting and refeeding [28]. Recently, Sukonina *et al.* [29] reported that ANGPTL4 regulates LpL activity by converting active LpL dimers to inactive monomers. In addition, the number of LpL binding sites at the surface of the capillary endothelium may be subject to metabolic regulation. Increased LpL activity in the heart in response to fasting is mediated in part by an increased number of LpL binding sites along the capillary endothelium [30].

LpL Deficiency Causes Chylomicronemia

A deficiency in either LpL or apo-CII in humans leads to chylomicronemia, with triglyceride levels >2,000 mg/dl. This syndrome can be associated with memory loss, abdominal pain and pancreatitis, dyspnea, and eruptive xanthomata [31,32]. In patients with LpL deficiency, the clearance of apo-B48- and apo-B100-containing lipoproteins from the plasma is markedly delayed, simply because their removal requires the hydrolysis of triglycerides by LpL [33].

Lpl^{-/-} mice appear normal at birth but die within 24 h [34,35], either from ischemia due to poor circulation or from starvation due to the inability to utilize lipid nutrients in milk. *Lpl*^{-/-} pups have low glucose levels and depleted tissue stores of triglycerides, and their plasma triglyceride levels are >20,000 mg/dl [34]. In one study, a small percentage of *Lpl*^{-/-} pups was rescued with an LpL adenovirus [36]. Even though the adenovirus-mediated expression of LpL was undetectable after a few weeks, the mice survived for up to 2 years. Interestingly, the rescued mice had plasma triglyceride levels of 2,000–4,000 mg/dl on a chow diet—very similar to those in chow-fed *Gpihbp1*^{-/-} mice described below.

A Deficiency in GPIHBP1 Also Causes Chylomicronemia

Recently, Beigneux and coworkers [37] reported severe chylomicronemia in mice lacking glycosylphosphatidylinositol-anchored high density lipoprotein-binding protein 1 (GPIHBP1). Both male and female *Gpihbp1*^{-/-} mice had grossly milky plasma—even when fed a low-fat chow diet (Figure 1), and many of the mice had plasma triglyceride levels of 2,000–4,000 mg/dl. The plasma cholesterol levels were also markedly elevated. The vast majority of the triglycerides and cholesterol in the plasma of *Gpihbp1*^{-/-} mice was in large lipoproteins, and the HDL cholesterol levels were low, as is generally the case with hypertriglyceridemia [31,36]. The plasma lipid levels in *Gpihbp1*^{+/-} and *Gpihbp1*^{+/+} mice were indistinguishable.

Prior to the discovery of chylomicronemia in *Gpihbp1*^{-/-} mice, there had only one publication on GPIHBP1. Ioka *et al.* [38] originally identified GPIHBP1, by expression cloning, as a molecule that conferred upon CHO cells the ability to bind fluorescently labeled HDL. The primary structure of GPIHBP1 consists of an amino-terminal signal sequence, a highly negatively charged domain (17 of 25 residues in the mouse sequence and 21 of 25 in the human sequence are glutamates or aspartates), an Ly-6 motif containing multiple cysteine residues, and a carboxyl-terminal hydrophobic motif that triggers the addition of a GPI anchor (Figure 2). The GPI anchor, which tethers GPIHBP1 to the cell surface, can be cleaved by a phosphatidylinositol-specific phospholipase C (PIPLC) [38]. Like SR-BI [39,40], GPIHBP1 was shown to mediate the selective uptake of lipids; unlike SR-BI, GPIHBP1 did not promote HDL-dependent cholesterol efflux. Excess unlabeled HDL reduced the binding of ¹²⁵I-HDL to GPIHBP1, and human apo-AI (lipid-free), phosphatidylserine, and acetylated LDL reduced

binding by ~50%. These findings led the authors to suggest that GPIHBP1 plays a role in cellular cholesterol transport [38].

Analyzing the Chylomicronemia Phenotype Associated with GPIHBP1 Deficiency

The triglyceride-rich lipoproteins in *Gpihbp1*^{-/-} mice were extremely large, far larger than in wild-type mice (Figure 3) [37]. The median diameter of lipoproteins was 157% larger in *Gpihbp1*^{-/-} mice than in *Gpihbp1*^{+/+} mice. Approximately 15% of the particles in *Gpihbp1*^{-/-} mice were immense, with diameters of 122–289 nm. The plasma lipoproteins of *Gpihbp1*^{-/-} mice contain increased amounts of apo-B48, as judged by a Coomassie blue-stained SDS-polyacrylamide gel and by a western blot with a mouse apo-B-specific monoclonal antibody [41].

The milky plasma in *Gpihbp1*^{-/-} mice, the large lipoproteins, and the increased plasma levels of apo-B48 strongly suggested a defect in the lipolytic processing of chylomicrons. To examine this possibility, Beigneux *et al.* [37] performed a retinyl palmitate clearance study. After oral administration of retinyl palmitate, retinyl esters are packaged into chylomicrons; their disappearance from the plasma reflects the rate of chylomicron clearance. In *Gpihbp1*^{-/-} mice, the peak retinyl ester levels were more than 10-fold higher than in *Gpihbp1*^{+/+} mice, and these high levels persisted for 24 h. In wild-type mice, the retinyl ester levels peaked by 1–3 h and had largely disappeared from the circulation by 10 h.

Unlike *Lpl*^{-/-} pups, *Gpihbp1*^{-/-} pups did not exhibit perinatal lethality. Beigneux *et al.* [37] examined the plasma lipid levels in suckling *Gpihbp1*^{-/-} and *Gpihbp1*^{+/+} mice; the *Gpihbp1*^{-/-} pups had far higher lipid levels ($P < 0.001$), but their triglyceride and cholesterol levels remained <250 mg/dl. In contrast, suckling *Lpl*^{-/-} pups were reported to have plasma triglyceride levels >20,000 mg/dl [34]. The mechanisms for the low lipid levels in *Gpihbp1*^{-/-} suckling mice are not understood, but it is known that the suckling phase is associated with high levels of *Lpl* expression in the liver [37,42]. It is tempting to speculate that the increased hepatic expression of LpL might be at the root of the relatively mild hypertriglyceridemia in young *Gpihbp1*^{-/-} mice, but this needs to be investigated.

GPIHBP1 Is Located on the Luminal Face of the Capillary Endothelium

The chylomicronemia in mice lacking a GPI-anchored cell-surface protein led Beigneux and coworkers [37] to hypothesize that GPIHBP1 is involved in the processing of triglyceride-rich lipoproteins by LpL along the surface of capillaries. In support of this concept, the pattern of GPIHBP1 expression in different tissues mirrored that of LpL [43]. In addition, immunofluorescence microscopy revealed that GPIHBP1 is expressed exclusively in endothelial cells of adipose tissue, heart, and skeletal muscle, colocalizing with the endothelial cell marker CD31 [37]. Confocal immunofluorescence microscopy showed that GPIHBP1 was located on the luminal face of capillaries (Figure 4) [37]. GPIHBP1 was virtually undetectable in the capillaries of the brain [37].

GPIHBP1 Binds Both LpL and Chylomicrons

Beigneux and coworkers [37] hypothesized that GPIHBP1 might be capable of binding LpL or chylomicrons or both. This hypothesis seemed plausible, as GPIHBP1 contains a strongly negatively charged domain, and LpL and several apolipoproteins within chylomicrons have positively charged domains that mediate protein–protein interactions [44,45]. To test this hypothesis, Beigneux and coworkers [37] constructed a full-length mouse *Gpihbp1* cDNA expression vector and expressed it in CHO cells lacking LDL receptor expression (CHO Id1A7).

The vector yielded high levels of GPIHBP1 expression on the surface of cells, and most of it could be released by treating the cells with PIPLC [46]. To assess the ability of GPIHBP1 to bind LpL, Beigneux and coworkers [37] examined the binding of avian LpL to CHO cells lacking the ability to synthesize heparan sulfate chains (pgsA-745 and pgsB-761 CHO) [47]. The cells transfected with *Gpihbp1* bound 10–20-fold more LpL than cells transfected with empty vector, and the binding was saturable, with only small amounts of nonspecific binding (Figure 5). The increased binding was eliminated by pretreating the cells with PIPLC (Figure 5). Parallel experiments showed that GPIHBP1 also binds to *human* LpL, and that the bound LpL could be released by either PIPLC or heparin [37].

To determine if GPIHBP1 binds chylomicrons, Beigneux and coworkers [37] isolated chylomicrons from the plasma of *Gpihbp1*^{−/−} mice, labeled them with the fluorescent dye DiI (1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate), and incubated them with CHO IdIA7 cells transfected with the mouse *Gpihbp1* cDNA or empty vector. The *Gpihbp1*-transfected cells bound chylomicrons avidly, and the binding was dramatically reduced by treating the transfected cells with PIPLC [37]. In transient transfection experiments, only a small percentage of cells expressed GPIHBP1, as judged by immunofluorescence microscopy, and *only* the GPIHBP1-expressing cells bound chylomicrons (Figure 6) [37].

The ability of GPIHBP1 to bind both to LpL and to chylomicrons suggests that GPIHBP1 draws chylomicrons and LpL into close proximity and serves as a “platform” for lipolysis (Figure 7) [37]. However, it is unlikely that *Gpihbp1* deficiency *eliminates* 100% of LpL-mediated lipolytic processing, for the simple reason that *Lpl*^{−/−} mice die shortly after birth with plasma triglyceride levels >20,000 mg/dl [31], whereas *Gpihbp1*^{−/−} mice survive the suckling period with far lower triglyceride levels. These phenotypic differences strongly suggest that some LpL-mediated lipolysis occurs in the absence of GPIHBP1, at least during the suckling period.

Residual LpL Activity in the Postheparin Plasma of *Gpihbp1*^{−/−} Mice

LpL activity in the plasma after an injection of heparin is reduced but not absent [37]. In multiple independent experiments with plasma samples from age- and sex-matched mice, LpL activity was 53.3%, 27.2%, 16.6%, and 68.4% lower in *Gpihbp1*^{−/−} mice than in wild-type mice [37]. LpL mass was also lower. LpL mass in the preheparin plasma of wild-type and *Gpihbp1*^{−/−} mice were low and not different. The fact that there was still some heparin-releasable LpL in *Gpihbp1*^{−/−} mice suggest that other molecules, perhaps HSPGs, could provide a reservoir for LpL binding (either inside the vasculature or in subendothelial spaces). It is conceivable that HSPGs provide a low-affinity but high-capacity binding site for LpL in the microvasculature. HSPGs could act as a trap for the newly synthesized LpL and also slow down chylomicrons in the bloodstream. GPIHBP1 might represent a higher affinity binding site, recruiting both LpL and chylomicrons from surrounding HSPGs, facilitating the hydrolysis of chylomicron triglycerides. The level of GPIHBP1 expression in tissues could prove to be one of the factors that regulate the rate of lipolysis of triglyceride-rich lipoproteins.

The Molecular Basis for Chylomicron Binding to GPIHBP1 Is Unknown

Chylomicrons contain several apolipoproteins with positively charged domains that bind to heparin sulfate and/or to HSPGs (*e.g.*, apo-B48, apo-AV apo-E) [48–50], so it is easy to imagine that one or more of these apolipoproteins mediate the binding of chylomicrons to the strongly negatively charged domain of GPIHBP1. Apo-AV is a good candidate as a ligand for GPIHBP1, since *Apoav* deficiency in mice causes hypertriglyceridemia associated with decreased LpL-mediated lipolysis [51–53]. In support of this idea, Beigneux and coworkers [37] reported increased binding of apo-AV phospholipid disks to cells that had been transfected with a *Gpihbp1* expression vector. Another interesting hypothesis is that GPIHBP1

acts as a receptor for apo-B48, the key structural protein of chylomicron particles. It would make sense that a receptor for apo-B48 would be located in the capillary endothelium of muscle and adipose tissue, where lipolysis occurs. The notion that apo-B48 might be a ligand for GPIHBP1 is also consistent with evolutionary considerations. Both apo-B48 and chylomicrons (defined as intestinal lipoproteins that are initially secreted into the lymph) are unique to mammals [54]. Similarly, GPIHBP1 is found in mammals (including platypus, see Figure 8) but is absent in lower organisms such as fish, amphibians, and birds [37]. The issue of the apolipoprotein ligand for GPIHBP1 requires further investigation.

Regulation of *Gpihbp1* Expression in Mice

Little is known about the regulation of GPIHBP1 expression. Beigneux and coworkers [37] reported that *Gpihbp1* mRNA levels were higher in the quadriceps of mice during fasting, and then returned to normal after refeeding a high-carbohydrate diet. However, it remains to be determined whether GPIHBP1 protein levels faithfully mirror the mRNA levels.

It is unclear why *Gpihbp1* is expressed highly in lipolytic tissues such as adipose tissue and heart, but apparently is virtually absent in the capillaries of the brain, which mainly relies on glucose for fuel. At this point, it is unclear whether this difference in endothelial cell gene expression is an intrinsic developmental property of endothelial cells in these tissues (*i.e.*, a property that would be sustained even after multiple passages in cell culture), or whether it depends on paracrine factors produced by the myocytes or adipocytes that surround the endothelial cells.

Conclusions

GPIHBP1 is an exciting new molecule in the lipolysis of triglyceride-rich lipoproteins, which is a central process in lipoprotein metabolism and in the delivery of lipid nutrients to tissues. GPIHBP1 is located on the luminal surface of the capillary endothelium and binds both LpL and chylomicrons; it likely forms a platform for lipolysis within capillaries. Elucidating the function and regulation of GPIHBP1 promises to shed light on the role of the endothelium in triglyceride metabolism in mammals.

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Figure 1. Plasma samples after low-speed centrifugation. On the right, lipemic plasma from a *Gpihbp1*-deficient mouse. The two samples on the left were from unaffected littermates. Reproduced, with permission from Elsevier, from the article by Beigneux and coworkers [37].

Figure 2.

Primary structure of mouse GPIHBP1. GPIHBP1 contains an amino-terminal signal peptide, a highly negatively charged domain (with 17 of 25 residues being glutamate or aspartate in the mouse protein), a Ly6 UPAR motif containing multiple cysteines, and a carboxyl-terminal hydrophobic motif triggering the addition of a glycosylphosphatidylinositol (GPI) anchor. A predicted site for N-glycosylation is shaded blue. The PIPLC cleavage site is indicated with a red arrow.

Figure 3. Electron micrographs of negatively stained $d < 1.006$ g/ml lipoproteins from the plasma of *Gpihbp1*^{-/-} and *Gpihbp1*^{+/+} mice, showing larger lipoproteins in *Gpihbp1*^{-/-} mice. Reproduced, with permission from Elsevier, from the article by Beigneux and coworkers [37].

Figure 4. GPIHBP1 is located within the lumen of the capillary endothelium of the heart. Confocal microscopy showing the binding of antibodies against CD31 and GPIHBP1 to heart tissue from a *Gpihbp1*^{+/+} mouse. GPIHBP1 staining is particularly prominent on the luminal face of the capillary endothelium. Images were taken with a 100× objective. Reproduced, with permission from Elsevier, from the article by Beigneux and coworkers [37].

Figure 5.

Binding of avian LpL (2.5 µg/ml) to pgsA-745 CHO cells transfected with pcDNA3-*Gpihbp1* or empty vector, before and after treatment with PIPLC. In parallel experiments, pgsB-761 cells transfected with the *Gpihbp1* cDNA also bound 10-fold more LpL than cells transfected with the empty vector. $P < 0.0001$ for all. Reproduced, with permission from Elsevier, from the article by Beigneux and coworkers [37].

Figure 6.

Binding of DiI-labeled chylomicrons (red) to nonpermeabilized CHO-IIdLA7 cells that had been transiently transfected with a mouse *Gpihbp1* cDNA. Binding was measured at 4°C. GPIHBP1 expression was detected with rabbit anti-GPIHBP1 antiserum and FITC-labeled anti-rabbit IgG (green). Reproduced, with permission from Elsevier, from the article by Beigneux and coworkers [37].

Figure 7. Schematic illustrating the binding of LpL and a chylomicron particle by GPIHBP1 on the surface of a capillary endothelial cell. Of note, the structural domains within GPIHBP1 that bind to LpL and chylomicrons have not yet been defined.

Homo sapiens MKALGAVLLALLLCGRPGRGOTQEEEEDEDHGPDYDEDE-DEVEEETNRLPGGR- 58
 Pan troglodytes MKALGAVLLALLLCGRPGRGOTQEEEEDEDHGPDYDEDE-DEVEEETNRLPGGR- 58
 Macaca mulatta MKVLRAVLLALLLCGQPGAGQVTLREPEELRKKEGMRPEP-AQSG-VDTNRLPGGR- 57
 Mus musculus MKALRAVLLILLSGQPGSGWAQ---EDGDADPEPENYNDDDDDEEEETNMIPGSRD 57
 Rattus norvegicus MKALRAVLLILLSGQPGSSWAQ---EAGDVDELEERYSYDDD--EEEEETNMIPGSRD 55
 Bos taurus MKALAAVLLALLWCRLQGRGRAQ---EEDDDPDAGREGYDDE--DEEEEAAGVPAGSRD 55
 Canis familiaris MTALPTVLLALLLCGRPGTGQGHPCGERGQLRRAQDKEDDDADFEDEEAASVNAAGRG 60
 Ornithorhynchus anat. LGALRSGLAGVVGKEVGPDPPEDEGEEEDYDEDELEYEAP-----TDGKRGIPIGRKE 55
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Homo sapiens SRVLLRCYCKSLPRDERCNLTQNC-SHGQCTTLIAHGNTESGLLTHSTWCTDSQPI 117
 Pan troglodytes SRGMA-----APTPEPC-----CLICLEHSGRESGLLTHSTWCTDSQPI 99
 Macaca mulatta GRVLLWCYTCQSLSRDEHCNLTQSC-SHGQACTTLIAHGNTESGLLTHSAWCTDNCQPI 116
 Mus musculus RAP-LQCYFCQVLHSGESCNETQSCSSSKPFCITLVSHSGTDKGYLTYSMWCTDTCQPI 116
 Rattus norvegicus RAPPLQCYFCQVLHSGESCNETQSCSSSKPFCITVISHGKTDGTGLTYSMWCTDTCQPI 115
 Bos taurus SGP--QCYTCQSLHKGESCEQVQSCVLPFG-TCKAIVSSWNTESGPQTYSWGCACTQAI 112
 Canis familiaris QAL-LRCYSCQSLHSGESCGRIQNCIRSHSFCKAVISHGNTESGFLTYSAWCADACKPI 119
 Ornithorhynchus anat. RGGELRCYTCESQFSKEFLNDTPCPSSSNCKTLISSGLTDSGHLTYSMWCADEFCEPL 115
 * * : : * : : * : : * : :

Homo sapiens TKTVEGTQVTMTCCQSSLCNVPPWQSSRVQDP----- 149
 Pan troglodytes TKTVEGTQVTMTCCQSSLCNVPPWQSSRVQDP----- 131
 Macaca mulatta TKTVEGTQVTMTCCQFNLNVPPWQSSRVQDP----- 148
 Mus musculus IKTVGQTQMTQTCQSTLCNIPPWQNPQVONPLGGRADSPLESGTRHPQGGKFSHPQVVK 176
 Rattus norvegicus VKTVDSQTQMTQTCQSTLCNIPPWQSPQIHNPLGGRADSPLESGTRHPQGGKFSHPQVVK 175
 Bos taurus SRTVDGSLTTISCCQSSLCNTPPWQDP----- 139
 Canis familiaris TKTLEGTMTLTCCQSSALCNLPWQDP----- 146
 Ornithorhynchus anat. TKTVHGTFMFVSCSSSLCGADSLDRKEE----- 144
 : * : : : * : : : :

Homo sapiens -----TGKGAGGPRGSSETVGAALLNLLAGLGAMGARRE 184
 Pan troglodytes -----TGKGAGGPRGSSETVGAALLNLLAGLGAMGARRE 166
 Macaca mulatta -----PGKGAGGPRGSSETAGTALLNLLAGLGAMGARRE 183
 Mus musculus AAHPQSDGANLPKSGKANPQGSAGYPSGWTGFGNIALLLSFFTCLEWASGA--- 228
 Rattus norvegicus VTHPQSDGAHLKSGKANPQGNAGFPAGWSKFGNVVLLTFLTSLWASGA--- 227
 Bos taurus -----QGRGAGFPQGSPTAVATVLLSLLASLQEMGL--- 171
 Canis familiaris -----LGRGAGFPQGDAMVATALLSLLPGLQAVGSSRE 181
 Ornithorhynchus anat. -----RDGAAAVAGGSLAALGASVLLGLL---AGL--- 172
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Figure 8.

Amino acid alignment showing the relatedness of GPIHBP1 in eight mammalian species. All sequences contain a stretch of negatively charged amino acids (red) at the amino terminus of the protein. Cysteines are in green. Predicted sites for N-glycosylation are shaded blue. The signal peptide is underlined. The carboxyl-terminal transmembrane domain is shaded gray.