Published in final edited form as:

Atherosclerosis. 2010 July ; 211(1): 1–8. doi:10.1016/j.atherosclerosis.2009.12.027.

THE METABOLISM OF TRIGLYCERIDE-RICH LIPOPROTEINS REVISITED; NEW PLAYERS, NEW INSIGHT

Geesje M. Dallinga-Thie^{1,2}, Remco Franssen², Hans L. Mooij², Maartje E. Visser², H. Carlijne Hassing², Frank Peelman³, John J.P. Kastelein², Miklós Péterfy^{4,5}, and Max Nieuwdorp²

¹Department of Experimental Vascular Medicine, Academic Medical Center, Amsterdam, the Netherlands ²Department of Vascular Medicine, Academic Medical Center, Amsterdam, the Netherlands ³Department of Medical Protein Research, VIB, Ghent University, Ghent, Belgium ⁴Department of Medicine, UCLA School of Medicine, Los Angeles, CA, USA ⁵Medical Genetics Institute, Cedars-Sinai Medical Center, Los Angeles, CA, USA

Abstract

Peripheral lipoprotein lipase (LPL)-mediated lipolysis of triglycerides is the first step in chylomicron/VLDL clearance involving heparan sulfate proteoglycans (HSPGs) displayed at the cell surface of the capillaries in adipose tissue, heart and skeletal muscle. The newly generated chylomicron remnant particles are then cleared by the liver, whereas VLDL remnant particles are either further modified, through the action of hepatic lipase (HL) and cholesteryl ester transfer protein (CETP), into LDL particles or alternatively directly cleared by the liver.

Two proteins, lipase maturation factor 1 (LMF1) and glycosylphosphatidylinositol-anchored high density lipoprotein binding protein 1 (GPIHBP1), have been recently identified and have revised our current understanding of LPL maturation and LPL-mediated lipolysis. Moreover, new insights have been gained with respect to hepatic remnant clearance using genetically modified mice targeting the sulfation of HSPGs and even deletion of the most abundant heparan sulfate proteoglycan: syndecan-1.

In this review, we will provide an overview of novel data on both peripheral TG hydrolysis and hepatic remnant clearance that will improve our knowledge of plasma triglyceride metabolism.

Keywords

triglycerides; proteoglycans; GPIHBP1; LMF1; lipoprotein lipase; NDST1; syndecan-1

Introduction

Recently, elevated plasma triglyceride levels have been shown to be an independent risk factor for cardiovascular disease.[1] It has long been recognized that lipoprotein lipase (LPL) is the essential enzyme involved in the lipolytic processing of triglycerides in chylomicrons and very low density lipoproteins (VLDL) in peripheral tissues.[2–6] Indeed, some patients with chylomicronemia have rare loss-of-function mutations in *LPL*, which explains the origin of the disease. However, in the majority of patients with

Correspondence should be addressed to, Geesje M. Dallinga-Thie, Department of Vascular Medicine and Laboratory of Vascular Medicine G1-113, Academic Medical Center, Meibergdreef 9, 1105AZ Amsterdam, the Netherlands. Phone: (0031) 20 5663224; g.m.dallinga@amc.nl.

chylomicronemia no mutations in *LPL* are identified, suggesting participation of other, yet unknown proteins in TG metabolism.

LPL is synthesized in tissue parenchymal cells, such as adipocytes and cardiomyocytes and then translocated to functional binding sites at the luminal surface of endothelial cells.[5,7] Heparan sulfate proteoglycans (HSPGs), located in lipid rafts at the endothelial cell surface in small capillaries of LPL secreting tissues are the docking stations for LPL; at this site, they provide a platform for interaction with triglyceride (TG)-rich lipoproteins by facilitating binding through the presence of excess negative charges on the surface of the HSPG molecule.[6,8–11] Once TG-rich lipoproteins are bound, LPL mediates TG hydrolysis, causing the release of fatty acids that are then taken up by receptors such as CD36 located on the plasma membrane of adipocytes and myocytes. Within these cells, the fatty acids are re-esterified and used for storage or they provide direct energy through mitochondrial oxidation.

The active form of LPL is a head-to-tail homodimer which may dissociate to produce inactive monomers.[12] Several effectors of LPL have been identified that modulate the *in vivo* function of LPL. One such important factor is apolipoprotein (apo)-CII, an essential co-factor for LPL activity, as evidenced by patients exhibiting severe chylomicronemia due to apo-CII deficiency. World-wide, only four mutations have been reported affecting the *APOC2* gene.[13] Mutations in *APOAV* have also been identified in patients with chylomicronemia, suggesting that apo-AV is important for proper LPL function.[13] However, in family studies, co-segregation of *APOAV* mutations with the hypertriglyceridemic trait is incomplete, suggesting that apo-AV deficiency alone is not sufficient to explain the observed hypertriglyceridemia.[14–16] Nevertheless, plasma levels of apo-AV are positively associated with plasma TG levels. [17,18] Finally, angiopoietin-like proteins (ANGPTL) 3 and 4 have also shown to inhibit LPL activity, resulting in increased plasma TG levels.[19–21] Consistent with its role as an inhibitor of LPL activity, rare loss-of-function mutations in *ANGPTL4* are mainly identified in individuals with low plasma TG levels.[20]

Recently, two new proteins have been identified that are involved post-translationally in regulating LPL activity. First, Lipase maturation factor 1 (LMF1) plays an essential role in the formation of catalytically active LPL from newly synthesized polypeptides in the endoplasmic reticulum (ER), a process called lipase maturation.[22] Mutations in the LMF1 gene in mice and humans cause combined lipase deficiency, affecting the enzyme activity for both LPL and hepatic lipase (HL), resulting in severe hypertriglyceridemia.[22] Second, Glycosylphosphatidylinositol-anchored high density lipoprotein binding protein 1 (GPIHBP1), is critically involved in LPL-mediated triglyceride hydrolysis of TG-rich lipoproteins, as illustrated by the severe chylomicronemia in the *Gpihbp1*^{-/-} mice.[23] Notably, we have identified a patient with chylomicronemia due to a homozygous mutation in *GPIHBP1* illustrating the importance of GPIHBP1 for LPL-mediated lipolysis in humans. [24]

Once TG-rich lipoproteins are hydrolyzed by LPL, the resultant chylomicron and VLDL remnants are cleared in the liver by the concerted action of receptor-mediated uptake involving members of the low density lipoprotein (LDL) receptor family or, as recently eloquently described, by direct interaction with HSPGs. [6,25–30] In this review we summarize recent data involving these new players in TG metabolism and hepatic clearance, LMF1, GPIHBP1 and the HSPGs.

Lipase Maturation Factor 1 (LMF1)

In 1983, a mouse was identified with a naturally occurring mutation on chromosome 17 resulting in severe chylomicronemia that caused neonatal death 2–3 days after birth.[31–33] Since activities of both LPL and HL were affected, this mutation was named combined lipase deficiency (*cld*). Surprisingly, lipase mRNA and protein levels were normal, implying that newly synthesized lipase polypeptides never attained enzyme activity, but rather remained retained in the ER as inactive, misfolded proteins.[34] The lack of lipase maturation in *cld* mice strongly supports the hypothesis that folding and assembly of lipases in the ER was aided by *trans*-acting factors, particularly the protein affected by the *cld* mutation.[7] The identification of this protein occurred in 2007, when a novel gene was identified that harbored the *cld* mutation.[22] In this study, it was shown that transmembrane protein 112 (*Tmem112*) was capable of restoring LPL activity to normal levels when transfected into mutant *cld* cells. To reflect its implied function, this gene was named Lipase Maturation Factor 1 (LMF1).[22]

LMF1 (NM_022773) is a membrane-bound protein localizing to the ER, the site of LPL and HL folding assembly.[22] It has 5 membrane-spanning regions, with its N-terminus located in the cytoplasm and the C-terminus within the ER lumen (Figure 1).[35] The amino-terminal domain and loop B and D are facing the cytoplasm, whereas loop A and C and the C-terminal domain are in the endoplasmic reticulum (ER) lumen. Initial studies have revealed that the function of lipase maturation resides in a domain of unknown function (DUF1222) that comprises about 65% of the C-terminus of LMF1 (loop B-D and the C-terminal domain as represented by the black line in Figure 1). [22] Truncation of this domain caused by the *cld* mutation results in loss of a C-terminal portion of the domain located within the ER- lumen, and result in the inability of LMF1 to support lipase maturation. The DUF1222 domain is present in over 50 different proteins among a wide variety of species. The importance of DUF1222 in *cld* suggests that its role may involve some form of protein maturation, perhaps as a client-specific chaperone or folding factor. Indeed, studies have indicated that loop C of the DUF1222 domain is essential for the interaction with nascent LPL and HL polypeptides in the ER, but cannot function independently of the C-terminal domain of LMF1.[35]

The human LMF1 gene is located on the short arm of chromosome 16, comprises 11 exons, and codes for a protein that contains 567 amino acids with a molecular weight of 65 kDa. Sequencing of the LMF1 gene in individuals with hypertriglyceridemia revealed several genetic variants. A homozygous nonsense mutation has been identified in exon 9 of the human LMF1 gene that results in an early stop codon (p.Y439X).[22] This patient suffers from severe chylomicronemia, pancreatitis and tubulous xanthomas as a result of a complete loss of active LPL. In addition, post-heparin plasma HL activity was decreased by 50%, recapitulating the combined lipase deficiency phenotype as seen in *cld* mice. A second homozygous nonsense mutation (p.W464X) has been identified in an individual with severe hypertriglyceridemia, recurrent pancreatitis and type 2 diabetes.[36] Postheparin LPL and HL activities in this patient were reduced 76% and 25%, respectively, indicating a somewhat milder phenotype compared to Y436X. In order to investigate the effect of LMF1 truncations, an in vitro assay was developed for the quantitative functional evaluation of LMF1 activity in lipase maturation.[37] Interestingly, whereas the Y439X variant was completely inactive, W464X retained ~40% of specific activity indicating a hypomorphic allele. Although both mutant messages are likely to be subject to nonsense-mediated RNA decay, it is tempting to speculate that residual activity of the W464Y protein may contribute to the milder phenotype in this patient. Based on the identification of the exact LPL interaction domain (Loop C) and the observation that the C-terminal domain is necessary to

have normal LMF1 function, we predict that only mutations that are located in these two domains will hamper LMF1 function and reduce LPL and HL activity in humans.

Many questions remain with regards to the precise function of LMF1. The phenotypic manifestations of the mouse and human mutations suggest a lipase-specific role for LMF1. Moreover, the evolutionary emergence of LMF1 and secreted dimeric lipases (i.e. LPL and HL) seems to coincide, suggesting an intimate functional relationship between these proteins.[22]. However, the tissue expression of LMF1 is ubiquitous and not restricted to only those tissues synthesizing LPL and HL, suggesting that LMF1 may have broader functions apart from lipase maturation that remain to be elucidated. [22] In support of this conclusion, splice variants of *LMF1* exist that are expressed and do not contain the DUF1222 domain.[35] Yet, the presence of these splice variants in a variety of tissues suggests a role for LMF1 other than lipase maturation. Indeed, more work needs to be done to understand the specific role of LMF1 in the context of the protein folding machinery in the ER.

GPIHBP1, A Novel Protein Involved in Triglyceride Lipolysis

When mature active LPL is secreted to the parenchymal cell surface it will bind to HSPGs. Since LPL exerts its function at the luminal site of vascular endothelial cells, a specific transcytotic transport mechanism via HSPGs or the VLDL receptor brings LPL to its site of action, where it is bound to cell surface HSPG.[38,39]. At least that was the general concept until in 2007 GPIHBP1 was identified as an essential platform for TG lipolysis in mice. [23,40] GPIHBP1 was originally identified by Ioka *et al* [41] as a protein involved in HDL binding. Remarkably, *Gpihbp1*^{-/-} mice manifest severe hyperchylomicronemia.[23] In mice, GPIHBP1 is located at the luminal site of the endothelial cell surface of small capillaries in skeletal muscle, adipose tissue and the heart, the same tissues that harbor LPL-mediated lipolysis.[23] Human GPIHBP1 is expressed in heart, adipose tissue and skeletal muscle, the same tissues that also express LPL (Figure 2). GPIHBP1 belongs to a large family of proteins that share a characteristic highly conserved lymphocyte antigen 6 (Ly-6) domain defined by the presence of 8 or 10 cysteine residues forming S-S bridges (Figure 3A) and providing the protein with the characteristic three finger motif (Figure 3B).[42] Other GPI-anchored proteins with the Ly-6 domain, such as CD59 and urokinase-type plasminogen activator receptor (uPAR) are N-glycosylated. The glycosylation in mouse GPIHBP1 is at Asn-76 within the Ly-6 domain, whereas human GPIHBP1 contains 2 glycosylation sites at Asn-76 and Asn-82 (Figure 3B).[43] Glycosylation of GPIHBP1 in the mouse is essential for trafficking of the protein to the cell surface. [43] GPIHBP1 is the only Ly-6 protein that has an additional highly negatively charged amino-terminal domain consisting of 25 amino acids of which 21 residues are glutamate or aspartate (Figure 3C). [23,41] GPIHBP1 plays an important role in TG hydrolysis by providing a binding site for LPL and chylomicrons. The presence of the acidic domain seems extremely well suited for protein-protein interactions since apolipoproteins and LPL both have very highly positively charged domains.[44–49] The acidic domain has proven to be essential for LPL binding in *in vitro* studies. In fact, replacement of the acidic residues completely eliminates the capacity of GPIHBP1 to bind LPL and chylomicrons.[50] Moreover, mutations in the heparin-binding domain of both LPL and apo-AV abolished the binding of these proteins to GPIHBP1. Based on these results one could predict that the acidic domain is the LPL binding domain of GPIHBP1. However, substitution of the Ly-6 domain from CD-59 for the Ly-6 domain of GPIHBP1 completely eliminated the ability of GPIHBP1 to bind LPL, even in the presence of the acidic domain [50]. These data suggest that both domains are essential to provide GPIHBP1 with the ability to bind LPL and chylomicrons. Further investigations using extensive molecular modeling experiments will have to be performed to fully explain the working mechanism.

The importance of the Ly-6 domain is underlined by the identification of the first human homozygous missense mutation located in a highly conserved region of the Ly-6 domain, a glutamine to a proline substitution (p.Q115P; Figure 3C).[24] The patient has life-long chylomicronemia and failure-to-thrive as a child but interestingly without signs of pancreatitis. Cell culture studies revealed that GPIHBP1-Q115P reaches the cell surface normally but the mutation completely eliminates the ability of GPIHBP1 to bind LPL and chylomicrons and show for the first time that GPIHBP1 is as critical for the lipolytic processing of lipoproteins in humans as it is in mice.[24] More recently, we identified a second homozygous missense mutation in a small child with severe chylomicronemia.[51,52] This second mutation, also located in the Ly-6 domain, changes the highly conserved cysteine residue at codon 65 to a tyrosine (p.C65Y) and results in a disruption of the first S-S double bond which may predict to result in a change in the fundamental structure of the Ly-6 domain (figure 3C). In line, a family was identified in northern Sweden in which three of the four children, with chylomicronemia, were compound heterozygote for two mutations in the Ly6 domain, p.C65S and p.C68G.[53] All patients showed a delayed LPL release after a heparin challenge, resulting in low levels of postheparin LPL mass and activity. The identification of these four mutations strongly supports the notion that GPIHBP1 is critical for TG lipolysis in humans as proposed in Figure 4. The importance of the Ly-6 cysteine molecules in proper LPL binding has been further illustrated *in vitro* using GPIHBP1 mutants for each of the 10 cysteine molecules.[54]

LPL can be released from its binding sites by heparin, as it is routinely used in the clinic to assess LPL activity.[55–57] LPL is present at the surface of endothelial cells where GPIHBP1 is expressed. However, *in vivo* co-localization has not been reported so far.. This pool of LPL will be released quickly into the plasma compartment after heparin injection. Additionally, LPL is also present in a subendothelial pool bound to HSPGs on the surface of adipocytes and myocytes.[6] Indeed, heparin injection in *Gpihbp1*^{-/-} mice show that the first quick release of LPL is completely absent, whereas there is a slow release of LPL at later time points. [58] This second subendothelial pool of LPL is irrelevant for TG hydrolysis, since *Gpihbp1*^{-/-} mice are hypertriglyceridemic.[58] More evidence in favor of GPIHBP1 being the major platform for TG hydrolysis is derived from experiments using an endothelial cell GlcNAc N-deacetylase/N-sulfotransferase deficient (*Ndst1*^{-/-}) mouse model. [58] NDST1 is required for the addition of sulfate groups to the structural proteoglycan protein in endothelial cells as well as in other tissues like the liver.[59,60] Surprisingly, inactivation of endothelial cell NDST1 did not result in elevated plasma TG levels. Moreover, postheparin LPL release was completely normal, adding to the notion that HSPG do not play an essential role in LPL-mediated TG lipolysis.[58] In contrast, postheparin LPL activity and mass in the GPIHBP1-Q115P proband was low, which may suggest that GPIHBP1 may be involved in the translocation of LPL to the endothelial cell surface in humans.[9,24] Interestingly, it has been proposed that GPIHBP1 may act as a stabilizer of the LPL protein and protects the LPL protein against de-activation by ANGPTL4 which is a known inhibitor of LPL activity.[19,21] Indeed, GPIHBP1-stabilized LPL is protected against deactivation by ANGPTL4. In line with these *in vitro* data is the observation that *Angptl4*^{-/-}/*Gpihbp1*^{-/-} mice have normal plasma TG levels.[21]

It has always been the question whether LPL deficiency itself is pro-atherogenic and leads to the development of atherosclerosis. Human data are scarce. Interestingly, LPL deficiency in mice aged 15 month results in increased formation of atherosclerotic lesions at the aortic root mediated by oxidation of chylomicrons lipids and induced activation of vascular endothelial cells.[61] In line with these observation is the identification of atherosclerosis in aged GPIHBP1 deficient mice.[62] It remains an intriguing question whether humans, deficient in LPL or GPIHBP1, develop increased atherosclerosis.

Role of Heparan Sulfate Proteoglycans in Hepatic Remnant Clearance (Figure 5)

The liver is the major organ for clearance of apoE-enriched TG-rich lipoprotein remnant particles. Cell surface receptors such as the LDL receptor (LDLr), LDL receptor-related protein 1 (LRP1) and HSPGs, are abundantly expressed on the surface of hepatocytes. In a concerted action together with apoE, LPL and HL they facilitate rapid hepatic clearance (Figure 5).[25,27,29,30,63,64] Chylomicron remnant particles penetrate the fenestrated endothelium in the liver and will subsequently directly interact with LDLr or will bind to HSPGs followed by internalize remnants directly or through LRP1.[6] The involvement of the LDL receptor in remnant clearance has been investigated some time ago using *LDLr*^{-/-} mice and in humans with a homozygous mutation in the LDLr gene.[65–67] These data unequivocally reveal that the LDL receptor is not rate-limiting in remnant clearance. In the absence of LDLr expression only a limited amount of remnant particles will accumulate. Moreover, patients with autosomal recessive hypercholesterolemia (ARH) develop hypercholesterolemia but VLDL clearance is normal, raising the possibility that the LDLr only binds VLDL remnants via apoE but internalization occurs through another cell surface component.[68] The contribution of LRP1 in remnant clearance has been demonstrated by Rohlmann *et al.*[28,69] Defective remnant clearance could only be found if both the LDLr and LRP1 were silenced. Of note, it has been shown that binding of apoE-enriched remnants to HSPGs in the Space of Disse allows final internalization through LRP1 (Figure 5). [25,27,28] Interestingly, Gordts *et al* [70] have shown that LRP1, defective in its endocytosis signal, leads to abnormal lipoprotein remnant clearance in the background of the absence of the LDLr. Although the TG accumulation was small, it provides subtle evidence that LRP1 may contribute to hepatic remnant clearance. Additionally, insulin may be involved in the translocation of LRP1 to the plasma membrane thereby favorably stimulating hepatic remnant clearance.[71]

Recently, the role of HSPG in hepatic remnant clearance has been boosted. Membrane bound hepatic HSPGs consist of a core protein, mainly syndecan1, with 3 heparan sulfate chains attached to it. [6,25,30,72] The *in vivo* role of HSPGs in remnant clearance was first illustrated by injecting heparinase, a heparan sulfate degrading enzyme, into the murine portal vein, which resulted in decreased VLDL and chylomicron remnant clearance.[73] Of note, the sulfate chains representing the amount of negative charges available on HSPG may differ between tissues and thereby provide specificity.[25] The highly sulfated HSPGs in the liver result in the negative charges that is crucial for the binding of LPL and apoE. To date, as many as 25 different enzymes have been discovered that participate in the synthesis of the mammalian HSPG sulfation pattern and the elongation of the heparan sulfate chain.[74] Tissue-specific knockout mouse models have recently been used to further investigate the role of hepatic HSPGs in remnant clearance. A hepatic *Ndst1*^{-/-} mice was developed, which is characterized by a strong decrease in sulfation of the HSPGs.[60] As expected, hepatic *Ndst1*^{-/-} mice show an increase in total plasma TG. This is mainly caused by an accumulation of TG in VLDL-like particles containing both apoB-100 and apoB-48. When the same mice are crossed to *LDLr*^{-/-} mice, a 7-fold increase in plasma TG was observed. In both models a delayed clearance of postprandial plasma lipoproteins was observed. Targeting of syndecan1 expression (*Sdc1*^{-/-}), which results in the absence of syndecan1 in hepatocytes, leads to elevation of plasma triglycerides due to accumulation of remnant particles.[72] Abundant expression of syndecan1 results in binding of VLDL remnant particles, but more importantly also facilitates degradation. Cross breeding with the hepatic *Ndst1*^{-/-} mice did not further increase plasma TG levels, indicating that syndecan1 is essential for the binding and clearance of TG-rich remnant lipoproteins. Interestingly, hepatic deficiency of HS2ST1, the enzyme involved in generating 2-O sulfated forms,

results in accumulation of plasma triglycerides due to impaired hepatic remnant clearance. [75] Thus specific sulfation forms are more important determinants for hepatic remnant clearance than overall negative charge.

In conclusion, the classical concept of TG metabolism requires a serious update since the identification of two novel proteins: LMF1 involved in maturation of LPL and GPIHBP1 in TG hydrolysis per se. Based on the identification of two homozygous mutations leading to a dysfunctional GPIHBP1 protein we conclude that GPIHBP1 provides a platform for TG hydrolysis in human as well as in mice. Additionally, novel data with the *Hs2st1*^{-/-}, *Ndst1*^{-/-} and *Sdc1*^{-/-} mice have forced us to conceive that hepatic remnant clearance should be recognized in the context of HSPGs present on the hepatic cell surface. Further investigations will improve our knowledge of both TG lipolysis and clearance and may help to develop novel therapies for hypertriglyceridemia.

Acknowledgments

We acknowledge the financial support of the Dutch Heart Association (2008-070B; RF) and Dutch Government ZonMW-VENI grant 2008 (016.096.044)(MN), National Institutes of Health Grant HL24841 and the Cedars-Sinai Medical Center (MP).

References

1. Nordestgaard BG, Benn M, Schnohr P, Tybjaerg-Hansen A. Nonfasting triglycerides and risk of myocardial infarction, ischemic heart disease, and death in men and women. *J. Am. Med. Ass.* 2007; 298:299–308.
2. Goldberg IJ, Merkel M. Lipoprotein lipase: physiology, biochemistry, and molecular biology. *Front Biosci.* 2001; 6:D388–D405. [PubMed: 11229871]
3. Bensadoun A. Lipoprotein lipase. *Annu. Rev. Nutr.* 1991; 11:217–237. [PubMed: 1892699]
4. Olivecrona T, Bengtsson-Olivecrona G. Lipoprotein lipase and hepatic lipase. *Curr. Opin. Lipidol.* 1993; 4:187–196.
5. Wang H, Eckel RH. Lipoprotein Lipase: from gene to obesity. *Am. J. Physiol Endocrinol. Metab.* 2009; 297:E271–E288. [PubMed: 19318514]
6. Williams KJ. Molecular processes that handle - and mishandle - dietary lipids. *J. Clin. Invest.* 2008; 118:3247–3259. [PubMed: 18830418]
7. Braun JEA, Severson DL. Regulation of the synthesis, processing and translocation of lipoprotein lipase. *Biochem. J.* 1992; 287:337–347. [PubMed: 1445192]
8. Goldberg IJ. Lipoprotein lipase and lipolysis: Central roles in lipoprotein metabolism and atherogenesis. *J Lipid Res.* 1996; 37:693–707. [PubMed: 8732771]
9. Williams KJ. Some things just have to be done in vivo: GPIHBP1, caloric delivery, and the generation of remnant lipoproteins. *Arterioscler. Thromb. Vasc. Biol.* 2009; 29:792–795. [PubMed: 19458350]
10. Sendak RA, Melford K, Kao A, Bensadoun A. Identification of the epitope of a monoclonal antibody that inhibits heparin binding of lipoprotein lipase: new evidence for a carboxyl-terminal heparin-binding domain. *J. Lipid Res.* 1998; 39:633–646. [PubMed: 9548595]
11. Parthasarathy N, Goldberg IJ, Sivaram P, Mulloy B, Flory DM, Wagner WD. Oligosaccharide sequences of endothelial cell surface heparan sulfate proteoglycan with affinity for lipoprotein lipase. *J. Biol Chem.* 1994; 269:22391–22396. [PubMed: 8071367]
12. Osborne JC Jr, Bengtsson-Olivecrona G, Lee NS, Olivecrona T. Studies on inactivation of lipoprotein lipase: role of the dimer to monomer dissociation. *Biochemistry.* 1985; 24:5606–5611. [PubMed: 4074716]
13. Franssen R, Visser ME, Kuivenhoven JA, Kastelein JJP, Dallinga-Thie GM, Stroes ES. Role of Lipoprotein Lipase in triglyceride metabolism: potential therapeutic target. *Future Lipidol.* 2008; 3:385–397.

14. Oliva CP, Pisciotto L, Li VG, Sambataro MP, Cantafora A, Bellocchio A, Catapano A, Tarugi P, Bertolini S, Calandra S. Inherited apolipoprotein A-V deficiency in severe hypertriglyceridemia. *Arterioscler. Thromb. Vasc. Biol.* 2005; 25:411–417.
15. Pullinger CR, Aouizerat BE, Movsesyan I, Durlach V, Sijbrands EJ, Nakajima K, Poon A, Dallinga-Thie GM, Hattori H, Green LL, Kwok PY, Havel RJ, Frost PH, Malloy MJ, Kane JP. An apolipoprotein A-V gene SNP is associated with marked hypertriglyceridemia among Asian-American patients. *J. Lipid Res.* 2008; 49:1846–1854. [PubMed: 18441017]
16. Talmud PJ. Rare APOA5 mutations--clinical consequences, metabolic and functional effects: an ENID review. *Atherosclerosis.* 2007; 194:287–292. [PubMed: 17222847]
17. Vaessen SF, Schaap FG, Kuivenhoven JA, Groen AK, Hutten BA, Boekholdt SM, Hattori H, Sandhu MS, Bingham SA, Luben R, Palmen JA, Wareham NJ, Humphries SE, Kastelein JJ, Talmud PJ, Khaw KT. Apolipoprotein A-V, triglycerides and risk of coronary artery disease: the prospective Epic- Norfolk Population Study. *J. Lipid Res.* 2006; 47:2064–2070. [PubMed: 16769999]
18. Dallinga-Thie GM, van Tol A, Hattori H, van Vark-van der Zee LC, Jansen H, Sijbrands EJ. Plasma apolipoprotein A5 and triglycerides in type 2 diabetes. *Diabetologia.* 2006; 49:1505–1511. [PubMed: 16752169]
19. Sukonina V, Lookene A, Olivecrona T, Olivecrona G. Angiopoietin-like protein 4 converts lipoprotein lipase to inactive monomers and modulates lipase activity in adipose tissue. *Proc. Natl. Acad. Sci. U.S.A.* 2006; 103:17450–17455. [PubMed: 17088546]
20. Romeo S, Yin W, Kozlitina J, Pennacchio LA, Boerwinkle E, Hobbs HH, Cohen JC. Rare loss-of-function mutations in ANGPTL family members contribute to plasma triglyceride levels in humans. *J. Clin. Invest.* 2009; 119:70–79. [PubMed: 19075393]
21. Sonnenburg WK, Yu D, Lee EC, Xiong W, Gololobov G, Key B, Gay J, Wilganowski N, Hu Y, Zhao S, Schneider M, Ding ZM, Zambrowicz BP, Landes G, Powell DR, Desai U. Glycosylphosphatidylinositol-anchored HDL-binding protein stabilizes lipoprotein lipase and prevents its inhibition by angiopoietin-like 3 and angiopoietin-like 4. *J. Lipid Res.* 2009; 50:2421–2429. [PubMed: 19542565]
22. Peterfy M, Ben-Zeev O, Mao HZ, Weissglas-Volkov D, Aouizerat BE, Pullinger CR, Frost PH, Kane JP, Malloy MJ, Reue K, Pajukanta P, Doolittle MH. Mutations in LMF1 cause combined lipase deficiency and severe hypertriglyceridemia. *Nat. Genet.* 2007; 39:1483–1487. [PubMed: 17994020]
23. Beigneux AP, Davies BS, Gin P, Weinstein MM, Farber E, Qiao X, Peale F, Bunting S, Walzem RL, Wong JS, Blazer WS, Ding ZM, Melford K, Wongsiriroj N, Shu X, De Sauvage FJ, Ryan RO, Fong LG, Bensadoun A, Young SG. Glycosylphosphatidylinositol-anchored high-density lipoprotein-binding protein 1 plays a critical role in the lipolytic processing of chylomicrons. *Cell Metab.* 2007; 5:279–291. [PubMed: 17403372]
24. Beigneux AP, Franssen R, Bensadoun A, Gin P, Melford K, Peter J, Walzem RL, Weinstein MM, Davies BS, Kuivenhoven JA, Kastelein JJ, Fong LG, Dallinga-Thie GM, Young SG. Chylomicronemia with a mutant GPIHBP1 (Q115P) that cannot bind lipoprotein lipase. *Arterioscler. Thromb. Vasc. Biol.* 2009; 29:956–962. [PubMed: 19304573]
25. Bishop JR, Stanford KI, Esko JD. Heparan sulfate proteoglycans and triglyceride-rich lipoprotein metabolism. *Curr. Opin. Lipidol.* 2008; 19:307–313. [PubMed: 18460924]
26. Bishop JR, Schuksz M, Esko JD. Heparan sulphate proteoglycans fine-tune mammalian physiology. *Nature.* 2007; 446:1030–1037. [PubMed: 17460664]
27. Mahley RW, Huang Y. Atherogenic remnant lipoproteins: role for proteoglycans in trapping, transferring, and internalizing. *J. Clin. Invest.* 2007; 117:94–98. [PubMed: 17200713]
28. Mahley RW, Ji ZS. Remnant lipoprotein metabolism: key pathways involving cell-surface heparan sulfate proteoglycans and apolipoprotein E. *J. Lipid Res.* 1999; 40:1–16. [PubMed: 9869645]
29. Fuki IV, Kuhn KM, Lomazov IR, Rothman VL, Tuszynski GP, Iozzo RV, Swenson TL, Fisher EA, Williams KJ. The syndecan family of proteoglycans. Novel receptors mediating internalization of atherogenic lipoproteins in vitro. *J. Clin. Invest.* 1997; 100:1611–1622. [PubMed: 9294130]

30. Mortimer BC, Martins I, Zeng BJ, Redgrave TG. Use of gene-manipulated models to study the physiology of lipid transport. *Clin. Exp. Pharmacol. Physiol.* 1997; 24:281–285. [PubMed: 9131298]
31. Paterniti JR Jr, Brown WV, Ginsberg HN, Artzt K. Combined lipase deficiency (cld): a lethal mutation on chromosome 17 of the mouse. *Science.* 1983; 221:167–169. [PubMed: 6857276]
32. Olivecrona T, Bengtsson-Olivecrona G, Chernick SS, Scow RO. Effect of combined lipase deficiency (cld/cld) on hepatic and lipoprotein lipase activities in liver and plasma of newborn mice. *Biochim. Biophys. Acta.* 1986; 876:243–248. [PubMed: 3955063]
33. Scow RO, Schultz CJ, Park JW, Blanchette-Mackie EJ. Combined lipase deficiency (cld/cld) in mice affects differently post-translational processing of lipoprotein lipase, hepatic lipase and pancreatic lipase. *Chem. Phys. Lipids.* 1998; 93:149–155. [PubMed: 9720257]
34. Reue K, Doolittle MH. Naturally occurring mutations in mice affecting lipid transport and metabolism. *J. Lipid Res.* 1996; 37:1387–1405. [PubMed: 8827513]
35. Doolittle MH, Neher SB, Ben-Zeev O, Ling-Liao J, Gallagher CM, Hosseini M, Yin F, Wong H, Walter P, Peterfy M. Lipase maturation factor 1 (Lmf1): Membrane topology and interaction with lipase proteins in the endoplasmic reticulum. *J. Biol Chem.* 2009
36. Cefalu AB, Noto D, Arpi ML, Yin F, Spina R, Hilden H, Barbagallo CM, Carrocio A, Tarugi P, Squatrito S, Vigneri R, Taskinen M-R, Peterfy M, Aversa MR. Novel LMF1 nonsense mutation in a patient with severe hypertriglyceridemia. *J. Clin Endocrinol. Metab.* 2009
37. Yin F, Doolittle MH, Peterfy M. A quantitative assay measuring the function of lipase maturation factor 1 (Lmf1). *J. Lipid Res.* 2009; 50:2265–2269. [PubMed: 19471043]
38. Obunike JC, Lutz EP, Li ZH, Paka L, Katopodis T, Strickland DK, Kozarsky KF, Pillarisetti S, Goldberg IJ. Transcytosis of lipoprotein lipase across cultured endothelial cells requires both heparan 21 sulfate proteoglycans and the very low density lipoprotein receptor. *J. Biol. Chem.* 2001; 276:8934–8941. [PubMed: 11121409]
39. Olivecrona, T.; Bengtsson-Olivecrona, G. Lipoprotein Lipase From Milk-the Model Enzyme in Lipoprotein Lipase Research. Borensztajn, J., editor. Chicago, Illinois: Lipoprotein Lipase; 1987. p. 15-58.
40. Young SG, Davies BS, Fong LG, Gin P, Weinstein MM, Bensadoun A, Beigneux AP. GPIHBP1: an endothelial cell molecule important for the lipolytic processing of chylomicrons. *Curr. Opin. Lipidol.* 2007; 18:389–396. [PubMed: 17620854]
41. Ioka RX, Kang MJ, Kamiyama S, Kim DH, Magoori K, Kamataki A, Ito Y, Takei YA, Sasaki M, Suzuki T, Sasano H, Takahashi S, Sakai J, Fujino T, Yamamoto TT. Expression cloning and characterization of a novel glycosylphosphatidylinositol-anchored high density lipoprotein-binding protein, GPI-HBP1. *J Biol. Chem.* 2003; 278:7344–7349. [PubMed: 12496272]
42. Ploug M, Ellis V. Structure-function relationships in the receptor for urokinase-type plasminogen activator. Comparison to other members of the Ly-6 family and snake venom alphaneurotoxins. *FEBS Lett.* 1994; 349:163–168. [PubMed: 8050560]
43. Beigneux AP, Gin P, Davies BS, Weinstein MM, Bensadoun A, Ryan RO, Fong LG, Young SG. Glycosylation of Asn-76 in mouse GPIHBP1 is critical for its appearance on the cell surface and the binding of chylomicrons and lipoprotein lipase. *J. Lipid Res.* 2008; 49:1312–1321. [PubMed: 18340083]
44. Lookene A, Beckstead JA, Nilsson S, Olivecrona G, Ryan RO. Apolipoprotein A-V-heparin interactions: implications for plasma lipoprotein metabolism. *J. Biol Chem.* 2005; 280:25383–25887. [PubMed: 15878877]
45. Sendak RA, Bensadoun A. Identification of a heparin-binding domain in the distal carboxylterminal region of lipoprotein lipase by site-directed mutagenesis. *J. Lipid Res.* 1998; 39:1310–1315. [PubMed: 9643364]
46. Hill JS, Yang D, Nikazy J, Curtiss LK, Sparrow JT, Wong H. Subdomain chimeras of hepatic lipase and lipoprotein lipase - Localization of heparin and cofactor binding. *J. Biol. Chem.* 1998; 273:30979–30984. [PubMed: 9812994]
47. Lookene A, Nielsen MS, Gliemann J, Olivecrona G. Contribution of the carboxy-terminal domain of lipoprotein lipase to interaction with heparin and lipoproteins. *Biochem. Biophys. Res. Commun.* 2000; 271:15–21. [PubMed: 10777674]

48. Beg OU, Uddin M, Siddiqi AR. Analysis of heparin-binding sites in human lipoprotein lipase using synthetic peptides. *J. Protein Chem.* 1998; 17:807–815. [PubMed: 9988527]
49. Hussain MM, Obunike JC, Shaheen A, Hussain MJ, Shelness GS, Goldberg IJ. High affinity binding between lipoprotein lipase and lipoproteins involves multiple ionic and hydrophobic interactions, does not require enzyme activity, and is modulated by glycosaminoglycans. *J. Biol. Chem.* 2000; 275:29324–29330. [PubMed: 10882743]
50. Gin P, Yin L, Davies BS, Weinstein MM, Ryan RO, Bensadoun A, Fong LG, Young SG, Beigneux AP. The acidic domain of GPIHBP1 is important for the binding of lipoprotein lipase and chylomicrons. *J. Biol. Chem.* 2008; 283:29554–29562. [PubMed: 18713736]
51. Dallinga-Thie GM, Beigneux AP, Franssen R, Hertecant J, Gin P, Weinstein MM, Kastelein JJ, Bensadoun A, Fong L, Young SG. Two mutations in GPIHBP1 cause severe chylomicronemia and provide evidence for a critical role of GPIHBP1 in human triglyceride metabolism. *Arterioscler. Thromb. Vasc. Biol.* 2009; 29:e10.
52. Franssen R, Young SG, Peelman F, Hertecant J, Sierts JA, Schimmel AWM, Bensadoun A, Kastelein JJP, Fong LG, Dallinga-Thie GM, Beigneux AP. Chylomicronemia with Low Postheparin Lipoprotein Lipase Levels in the Setting of GPIHBP1 Defects. *Circulation Cardiovasc. Genet.* 2010 in press.
53. Olivecrona G, Ehrenborg E, Semb H, Makoveichuk E, Lindberg A, Hayden MR, Gin P, Davies BSJ, Weinstein MM, Fong L, Beigneux AP, Young SG, Olivecrona T, Hernell O. Mutation of Conserved Cysteines in the LY6 Domain of GPIHBP1 in Familial Chylomicronemia. *J. Lipid Res.* 2010 in press.
54. Beigneux AP, Gin P, Davies BS, Weinstein MM, Bensadoun A, Fong LG, Young SG. Highly conserved cysteines within the Ly6 domain of GPIHBP1 are crucial for the binding of lipoprotein lipase. *J. Biol. Chem.* 2009; 284:30240–30247. [PubMed: 19726683]
55. Korn ED. Clearing factor, a heparin-activated lipoprotein lipase. II. Substrate specificity and activation of coconut oil. *J. Biol. Chem.* 1955; 215:15–26. [PubMed: 14392138]
56. Babirek SP, Iverius PH, Fujimoto WY, Brunzell JD. Detection and characterization of the heterozygote state for lipoprotein lipase deficiency. *Arterioscler. Thromb.* 1989; 9:326–334.
57. Olivecrona, T.; Olivecrona, G. Determination and Clinical Significance of Lipoprotein Lipase and Hepatic Lipase. In: Rifai, N.; Warnick, GR.; Dominiczak, M., editors. *Handbook of lipoprotein testing*. Washington DC: AACC press; 2000. p. 479-497.
58. Weinstein MM, Yin L, Beigneux AP, Davies BS, Gin P, Estrada K, Melford K, Bishop JR, Esko JD, Dallinga-Thie GM, Fong LG, Bensadoun A, Young SG. Abnormal patterns of lipoprotein lipase release into the plasma in GPIHBP1-deficient mice. *J. Biol. Chem.* 2008; 283:34511–34518. [PubMed: 18845532]
59. Wang L, Fuster M, Sriramaraio P, Esko JD. Endothelial heparan sulfate deficiency impairs L-selectin- and chemokine-mediated neutrophil trafficking during inflammatory responses. *Nat. Immunol.* 2005; 6:902–910. [PubMed: 16056228]
60. MacArthur JM, Bishop JR, Stanford KI, Wang L, Bensadoun A, Witztum JL, Esko JD. Liver heparan sulfate proteoglycans mediate clearance of triglyceride-rich lipoproteins independently of LDL receptor family members. *J. Clin. Invest.* 2007; 117:153–164. [PubMed: 17200715]
61. Zhang X, Qi R, Xian X, Yang F, Blackstein M, Deng X, Fan J, Ross C, Karasinska J, Hayden MR, Liu G. Spontaneous atherosclerosis in aged lipoprotein lipase-deficient mice with severe hypertriglyceridemia on a normal chow diet. *Circ. Res.* 2008; 102:250–256. [PubMed: 18032735]
62. Weinstein MM, Yin L, Tu Y, Wang X, Wu X, Castellani LW, Walzem RL, Lusis AJ, Fong LG, Beigneux AP, Young SG. Chylomicronemia Elicits Atherosclerosis in Mice. *Arterioscler. Thromb. Vasc. Biol.* 2009
63. Mahley RW, Ji ZS. Remnant lipoprotein metabolism: key pathways involving cell-surface heparan sulfate proteoglycans and apolipoprotein E. *J. Lipid Res.* 1999; 40:1–16. [PubMed: 9869645]
64. Heeren J, Beisiegel U, Grewal T. Apolipoprotein E recycling: implications for dyslipidemia and atherosclerosis. *Arterioscler. Thromb. Vasc. Biol.* 2006; 26:442–448. [PubMed: 16373604]
65. Herz J, Qiu S-Q, Oesterle A, DeSilva HV, Shafi S, Havel RJ. Initial hepatic removal of chylomicron remnants is unaffected but endocytosis is delayed in mice lacking the low density lipoprotein receptor. *Proc. Natl. Acad. Sci. USA.* 1995; 92:4611–4615. [PubMed: 7753850]

66. Rubinsztein DC, Cohen JC, Berger GM, Westhuyzen DR, der van, Coetzee GA, Gevers W. Chylomicron remnant clearance from the plasma is normal in familial hypercholesterolemic homozygotes with defined receptor defects. *J. Clin. Invest.* 1990; 86:1306–1312. [PubMed: 2212015]
67. Kita T, Goldstein JL, Brown MS, Watanabe Y, Hornick CA, Havel RJ. Hepatic uptake of chylomicron remnants in WHHL rabbits: a mechanism genetically distinct from the low density lipoprotein receptor. *Proc Natl. Acad. Sci. U.S.A.* 1982; 79:3623–3627. [PubMed: 6285353]
68. Jones C, Garuti R, Michaely P, Li WP, Maeda N, Cohen JC, Herz J, Hobbs HH. Disruption of LDL but not VLDL clearance in autosomal recessive hypercholesterolemia. *J. Clin. Invest.* 2007; 117:165–174. [PubMed: 17200716]
69. Rohlmann A, Gotthardt M, Hammer RE, Herz J. Inducible inactivation of hepatic LRP gene by Cre-mediated recombination confirms role of LRP in clearance of chylomicron remnants. *J. Clin. Invest.* 1998; 101:689–695. [PubMed: 9449704]
70. Gordts PL, Reekmans S, Lauwers A, Van DA, Verbeek L, Roebroek AJ. Inactivation of the LRP1 intracellular NPxYxxL motif in LDLR-deficient mice enhances postprandial dyslipidemia and atherosclerosis. *Arterioscler. Thromb. Vasc. Biol.* 2009; 29:1258–1264. [PubMed: 19667105]
71. Laatsch A, Merkel M, Talmud PJ, Grewal T, Beisiegel U, Heeren J. Insulin stimulates hepatic low density lipoprotein receptor-related protein 1 (LRP1) to increase postprandial lipoprotein clearance. *Atherosclerosis.* 2009; 204:105–111. [PubMed: 18834984]
72. Stanford KI, Bishop JR, Foley EM, Gonzales JC, Niesman IR, Witztum JL, Esko JD. Syndecan-1, the primary heparan sulfate proteoglycan mediating hepatic triglyceride-rich lipoprotein clearance in vivo. *J. Clin. Invest.* 2009; 119:3236–3245. [PubMed: 19805913]
73. Ji ZS, Sanan DA, Mahley RW. Intravenous heparinase inhibits remnant lipoprotein clearance from the plasma and uptake by the liver: in vivo role of heparan sulfate proteoglycans. *J. Lipid Res.* 1995; 36:583–592. [PubMed: 7539827]
74. Esko JD, Selleck SB. Order out of chaos: assembly of ligand binding sites in heparan sulfate. *Annu. Rev. Biochem.* 2002; 71:435–471. [PubMed: 12045103]
75. Stanford KI, Wang L, Castagnola J, Song D, Bishop JR, Brown JR, Lawrence R, Bai X, Habuchi H, Tanaka M, Cardoso WV, Kimata K, Esko JD. Heparan sulfate 2-O-sulfotransferase is required for triglyceride-rich lipoprotein clearance. *J. Biol Chem.* 2009
76. Kersten S, Lichtenstein L, Steenbergen E, Mudde K, Hendriks HF, Hesselink MK, Schrauwen P, Muller M. Caloric restriction and exercise increase plasma ANGPTL4 levels in humans via elevated free fatty acids. *Arterioscler. Thromb. Vasc. Biol.* 2009; 29:969–974. [PubMed: 19342599]
77. Kjaergaard M, Hansen LV, Jacobsen B, Gardsvoll H, Ploug M. Structure and ligand interactions of the urokinase receptor (uPAR). *Front Biosci.* 2008; 13:5441–5461. [PubMed: 18508598]
78. Jacobsen B, Ploug M. The urokinase receptor and its structural homologue C4.4A in human cancer: expression, prognosis and pharmacological inhibition. *Curr. Med. Chem.* 2008; 15:2559–2573. [PubMed: 18855679]
79. Galat A. The three-fingered protein domain of the human genome. *Cell Mol. Life Sci.* 2008; 65:3481–3493. [PubMed: 18821057]

Figure 1. Protein Structure of LMF1

LMF1 contains 5 membrane-spanning regions, with its N-terminus located in the cytosol and the C-terminus within the ER lumen. The function of lipase maturation resides in a domain of unknown function (DUF1222, indicated by black line) that comprises about 65% of the C-terminus of LMF1. Partial truncations of this domain caused by *cld*, Y439X and W464X result in loss of a C-terminal portion of the domain located within the ER lumen, and result in impaired activity of LMF1 to support lipase maturation. The lipase interaction site and a glycosylation site at residue 430 are indicated

Figure 2. Tissue expression profile of GPIHBP1 and LPL

The expression profile of GPIHBP1 and LPL mRNA in human adipose tissue, heart and skeletal muscle (first choice human total RNA Survey panel, Ambion, Foster City, Ca, USA).[76] The mRNA was reversed transcribed and used for qPCR of GPIHBP1 and LPL. 36B4 is used as a house keeping gene.

Figure 3. A Model for GPIHBP1

Figure 3 A shows the consensus sequence of the Ly-6 motif.[77,78] The conserved cysteine residues are presented in yellow, whereas the non-conserved cysteine residues are shown in white. GPIHBP1 consists of 10 cysteine residues in the Ly-6 domain which will form 5 S-S bonds that determine its characteristic three-finger structure.[79] The consensus sequence of CD59 is very similar to GPIHBP1. The model has been build using the loop model procedure of MODELER 9v6. The model with the best DOPE (Discrete Optimized Protein Energy) score was selected. Based on this model we predict the structure of GPIHBP1 as shown in figure 3B. The cysteine residues are located in the global core of the protein. The three loops are formed based on the presence of 6 β -strands. Additionally the two putative glycosylation sites (Asn-76 and Asn-82) are shown. The p.Q115P is located adjacent to the 7th cysteine residue, whereas the p.C65Y mutation is the first cysteine residue of the Ly-6 domain. We hypothesize that both mutations may interfere with proper folding of the Ly-6 domain and consequently with the interaction between the acidic and the Ly-6 domain.

Figure 4. Peripheral TG Hydrolysis

LPL is synthesized in parenchymal cells, matured by LMF1 and secreted in the subendothelial space where it binds to syndecan1. LPL will then be transcytosed through the endothelial cell to reach the cell surface at the luminal site where GPIHBP1 is present, probably in lipid rafts together with syndecan1 and fatty acid transporters such as CD36. [6,9] The generated remnant particle can then go to the liver. The fatty acids will be locally internalized by CD36.

Figure 5. Hepatic Remnant Clearance

View of hepatocytes with sinusoids and bile canaliculi. View of hepatocytes, with sinusoids and bile canaliculi (green). Hepatic remnant clearance involves sequestration of the particle through the fenestrae. On its journey through the extracellular matrix the remnant particle will acquire additional apoE molecules which enables rapid binding to the LDL receptor or syndecan1 receptor, the most abundant HSPG in hepatocytes, which internalizes remnant particles.[72] LRP1 only facilitates uptake after modification of the particle by syndecan1. Both the LDLr and syndecan1 contribute equally to remnant clearance.