Chylomicronemia With Low Postheparin Lipoprotein Lipase Levels in the Setting of GPIHBP1 Defects

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Methods and Results—We report the discovery of a homozygous missense mutation in GPIHBP1 in a young boy with severe chylomicronemia. The mutation, p.C65Y, replaces a conserved cysteine in the GPIHBP1 lymphocyte antigen 6 domain with a tyrosine and is predicted to perturb protein structure by interfering with the formation of a disulfide bond. Studies with transfected Chinese hamster ovary cells showed that GPIHBP1-C65Y reaches the cell surface but has lost the ability to bind lipoprotein lipase (LPL). When the GPIHBP1-C65Y homozygote was given an intravenous bolus of heparin, only trace amounts of LPL entered the plasma. We also observed very low levels of LPL in the postheparin plasma of a subject with chylomicronemia who was homozygous for a different GPIHBP1 mutation (p.Q115P). When the GPIHBP1-Q115P homozygote was given a 6-hour infusion of heparin, a significant amount of LPL appeared in the plasma, resulting in a fall in the plasma triglyceride levels from 1780 to 120 mg/dL.

Conclusions—We identified a novel GPIHBP1 missense mutation (p.C65Y) associated with defective LPL binding in a young boy with severe chylomicronemia. We also show that homozygosity for the C65Y or Q115P mutations is associated with low levels of LPL in the postheparin plasma, demonstrating that GPIHBP1 is important for plasma triglyceride metabolism in humans. (Circ Cardiovasc Genet. 2010;3:169-178.)

Key Words: lipoprotein lipase • GPIHBP1 • triglycerides

Clinical Perspective on p 178

During the past year, evidence for the involvement of GPIHBP1 in lipolysis has accumulated. Weinstein et al showed that LPL release into the plasma after an injection of heparin is abnormal in Gpihbp1−/− mice. After an intravenous injection of 50 U of heparin into Gpihbp1−/− mice, the total amount of LPL entering the plasma was virtually normal, but the LPL entered the plasma with delayed kinetics. This observation led to the speculation that the intravascular pool of LPL might be abnormally low in the setting of GPIHBP1 deficiency. Interestingly, the LPL that entered the plasma of Gpihbp1−/− mice after multiple intravenous injections of heparin was enzymatically active and quickly lowered plasma triglyceride levels. More recently, Sonnenburg et al made 2 other important observations: LPL is stabilized when it is bound to GPIHBP1 and is more resistant to inactivation by angiopoietin-like protein 4.

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Beigneux et al.8 provided the first evidence that GPIHBP1 is important for the processing of triglyceride-rich lipoproteins in humans. They identified a homozygous missense mutation in GPIHBP1 (p.Q115P) in a young man with lifelong chylomicronemia. Using several different cell culture-based assays, they showed that this glutamine-to-proline substitution nearly abolished the ability of the GPIHBP1 to bind LPL.

In the current studies, we have continued to investigate the importance of GPIHBP1 for triglyceride metabolism in humans. We show that homozygosity for a GPIHBP1 missense mutation, p.C65Y, causes chylomicronemia. Like the Q115P mutation, the C65Y mutation abolishes the ability of GPIHBP1 to bind LPL. We also show that the postheparin plasma LPL levels are abnormally low in humans with functionally defective GPIHBP1 proteins.

Methods

Subjects
A 3-year-old boy living in the United Arab Emirates was given a diagnosis of chylomicronemia. The exons of LPL, apolipoprotein A5 (APOA5), apolipoprotein C2 (APOC2), lipase maturation factor 1 (LMF1), and GPIHBP1 were amplified and sequenced. Studies on the patient’s family also were performed, and we included in our studies a young man with chylomicronemia who was homozygous for the Q115P mutation in GPIHBP1.8 Four healthy normolipidemic men and women were included as control subjects for the postheparin LPL study. Studies were approved by the Committees on Human Research at Academic Medical Center (Amsterdam, The Netherlands), the University of California, Los Angeles, and Tawam Hospital (Al Ain, United Arab Emirates). All subjects gave informed consent.

Genomic DNA Analyses
Genomic DNA was prepared from blood leukocytes. The exons of GPIHBP1, LPL, APOA5, and APOC2 (along with ~50 bp of flanking introns) were enzymatically amplified as described.9 To facilitate DNA sequencing, an M13 tail was added to each primer (forward, 5'-GTGTGGACCAATGGCTTGTCTG-3'; reverse, 5'-CAGAGCAACCCCTTTAAG-3'). All amplicons were sequenced in both directions.

Biochemical Measurements
Blood was collected after a 12-hour fast and was placed on ice. Plasma was isolated by centrifugation and stored at −80°C. Total
plasma cholesterol, triglycerides, high-density lipoprotein (HDL) cholesterol, and low-density lipoprotein (LDL) cholesterol levels were measured with commercial kits (Wako and Randox). Plasma apolipoprotein B (apoB), apoCII, and apoCIII levels were measured with commercial assays (Randox). Plasma apoB48 levels were determined with an ELISA (Shibayagi). Size fractionation of plasma lipoproteins was performed by fast protein liquid chromatography; online triglyceride measurements were obtained with a commercial assay (Biomerieux).8,9 Before the fractionation of fast protein liquid chromatography, the most buoyant lipoproteins in the proband’s plasma were removed by centrifugation at 10,000 rpm for 10 minutes at 4°C (to prevent clogging of the column). Plasma LPL levels were measured after an intravenous injection of heparin (molecular weight, 6500 Da [Leo Pharma]; 50 IU/kg body weight). Blood was collected into heparin-lithium tubes at baseline and 1, 2, 3, 6, 9, 12, 15, and 18 minutes after heparin and put on ice. LPL and hepatic lipase (HL) activity levels were measured as previously described.10 HL activity was measured after inhibiting LPL activity with a monoclonal antibody against human LPL (5D2) (a gift from Dr John Brunzell, University of Washington, Seattle, Wash) for 2 hours at 4°C, and 1 mU is equivalent to 1 nmol fatty acid released per minute. Plasma LPL and HL mass levels were measured with an ELISA (LPL, Daiichi; HL, as described by Bensadoun et al11). Heparin infusion studies were performed as described earlier, with a bolus injection of 2280 U heparin/m² followed by a continuous infusion of heparin (1984 U/m²/h).12 After collecting blood samples, tetrahydro-lipstatin was added to block lipolysis, and LPL mass and triglycerides were measured.

### GPIHBP1 Constructs and Cell Transfections

A human GPIHBP1 expression vector containing an internal S-protein tag, as well as mutant GPIHBP1 expression vectors with Q115P and G56R mutations have been described previously.4,8,13 In this study, we used the QuickChange kit (Stratagene) to introduce the C65Y mutation into the human GPIHBP1 expression vector. Transient transfections of Chinese hamster ovary (CHO) pgs-745 cells14 were performed by electroporation with the Nucleofector II apparatus (Lonza).

### Western Blots

Proteins were size fractionated on 4% to 12% Bis-Tris sodium dodecyl sulfate-polyacrylamide gels and electrophoretically transferred to nitrocellulose membranes. The antibody dilutions for Western blots were 1:1000 for a goat polyclonal antibody against the S-protein tag (Abcam); 1:100 for a mouse monoclonal antibody against human GPIHBP1; 1:200 for a mouse monoclonal antibody against the V5 tag (Invitrogen); 1:500 for a rabbit polyclonal antibody against actin (Abcam); 1:5000 for an IRdye800-conjugated donkey antigoat IgG (Li-Cor); 1:2000 for an IRdye680-conjugated donkey antirabbit IgG (Li-Cor); and 1:500 for an

### Table. Fasting Plasma Lipid and Apolipoprotein Levels

<table>
<thead>
<tr>
<th>Lipid and Apolipoprotein Levels</th>
<th>C65Y Homozygote</th>
<th>Father</th>
<th>Mother</th>
<th>Siblings (n=3)*</th>
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</thead>
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<tr>
<td>Triglycerides</td>
<td>1575</td>
<td>147</td>
<td>94</td>
<td>65±14</td>
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<tr>
<td>Total cholesterol</td>
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<td>217</td>
<td>129±12</td>
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<td>19.5</td>
<td>35.5</td>
<td>73</td>
<td>47.9±6.7</td>
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<tr>
<td>ApoB</td>
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<td>102</td>
<td>93</td>
<td>54±3</td>
</tr>
<tr>
<td>ApoB48, μg/mL</td>
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<td>2.1</td>
<td>2.6</td>
<td>5.2±0.8</td>
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<tr>
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<td>1.7±1.3</td>
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<tr>
<td>ApoCIII</td>
<td>34.5</td>
<td>8.2</td>
<td>9.1</td>
<td>6.3±1.1</td>
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</tbody>
</table>

All data are expressed as mg/dL unless otherwise indicated.

*Data are presented as mean±SD.

Figure 2. Detection of the GPIHBP1–C65Y mutant at the cell surface. CHO pgsA-745 cells were electroporated with an empty vector or an expression vector for wild-type human GPIHBP1, GPIHBP1–C65Y, GPIHBP1–C65A,16 GPIHBP1–Q115P, or GPIHBP1–G56R. All constructs contained an amino-terminal S-protein tag. The expression of GPIHBP1 was determined in nonpermeabilized cells (A) and in permeabilized cells (B) by immunofluorescence microscopy with a fluorescein isothiocyanate-conjugated goat antiserum against the S-protein tag. Cell nuclei were visualized with 4’,6-diamidino-2-phenylindole (blue).
IRdye800-conjugated donkey antimouse IgG (Li-Cor). Antibody binding was detected with an Odyssey infrared scanner (Li-Cor).

Assessing the Ability of GPIHBP1 to Reach the Cell Surface

To assess GPIHBP1 expression at the cell surface, we used 3 approaches. First, fluorescence microscopy was used to detect GPIHBP1 at the surface of CHO pgsA-745 cells that had been transfected with wild-type and mutant GPIHBP1 constructs. For these studies, CHO pgsA-745 cells were electroporated with 2.0 μg of plasmid DNA and plated on coverslips in 24-well plates. The cells were fixed in 3.0% paraformaldehyde, blocked with blocking buffer (phosphate-buffered saline [PBS] containing 1 mmol/L MgCl₂, 1 mmol/L CaCl₂, and 10% donkey serum), and incubated with a fluorescein isothiocyanate-conjugated goat antiserum against the S-protein tag. Actin was used as a loading control.

Second, we assessed the release of GPIHBP1 from the surface of transfected cells after they had been incubated with a phosphatidylinositol-specific phospholipase C (PIPLC). CHO pgsA-745 cells (1×10⁶ cells) were electroporated with 2.0 μg of plasmid DNA and seeded into duplicate wells of a 24-well plate. The next day, the cells were incubated with PIPLC (5 U/mL) in serum-free medium for 1 hour at 37°C. At the end of the incubation, the medium was harvested, and cell extracts were prepared. To assess the amount of GPIHBP1 on the cell surface, Western blots were performed on cell extracts with an IRdye680-conjugated donkey antigoat IgG (Li-Cor, 1:800). To assess the total amount of GPIHBP1 in cells, we simultaneously performed a Western blot with a mouse monoclonal antibody against GPIHBP1 (1:100). The binding of the mouse monoclonal antibody to GPIHBP1 was detected with an IRdye800-conjugated donkey antimouse IgG (Li-Cor, 1:2000). The ratio of GPIHBP1 on the cell surface (680-nm channel) compared with total GPIHBP1 (800-nm channel) was determined for each GPIHBP1 construct and expressed as a percentage of the ratio observed with wild-type GPIHBP1.

Assessing the Ability of GPIHBP1 to Bind LPL

To assess LPL binding to GPIHBP1, we incubated GPIHBP1-expressing cells with a V5-tagged human LPL. After washing the cells, we used Western blots to assess the amount of LPL bound to cells. For these studies, 5×10⁶ CHO pgsA-745 cells were electroporated with 5.0 μg of plasmid DNA and seeded into triplicate wells of a 24-well tissue culture plate. Twenty-four hours after the electroporation, cells were incubated for 2 hours at 4°C with V5-tagged human LPL (400 μL/well). In some wells, heparin (final concentration, 500 U/mL) was included in the incubation medium. At the end of the incubation period, cells were washed 6 times in ice-cold PBS/Mg/Ca, and the cell extracts were collected in radioimmunoprecipitation assay buffer containing complete mini EDTA-free protease inhibitors (Roche). The amounts of LPL and GPIHBP1 in cell extracts were assessed by Western blotting.

Results

A Novel Missense Mutation Involving a Highly Conserved Cysteine in GPIHBP1

A 3-year-old boy with chylomicronemia was examined. The patient was noted to have chylomicronemia at 1 year of age during a bout of pancreatitis. The fasting plasma triglyceride levels were 4005 mg/dL (45 mmol/L). On physical examination, the child was developing normally, but his body weight was less than the 10th percentile for his age. Fundoscopy revealed lipemia retinalis, there was no hepatosplenomegaly, and there were no eruptive xanthomas.

Sequence variations in LPL, APOC2, LMF1, and APOA5 were excluded by sequencing all the exons of those genes. Sequencing of the coding regions of GPIHBP1 led to the identification of a homozygous G-to-A transversion in exon 3 (c.194G>A), a mutation that changed a conserved cysteine at residue 65 to a tyrosine (p.C65Y) (Figure 1A). The presence of this mutation was confirmed by restriction endonuclease digestion of a DNA fragment amplified from the genomic DNA of the C65Y homozygote (Figure 1B). The proband’s hyperlipidemia was partially responsive to diet, as is generally the case with patients with chylomicronemia. The plasma triglyceride level fell from ∼4000 to 1575 mg/dL (17.7 mmol/L) when he was placed on a low-fat diet. As expected, fractionation of the plasma by fast protein liquid chromatography revealed that the vast majority of the triglycerides were in large lipoproteins (Figure 1C). Plasma apoB48
levels were elevated, as were plasma levels of apoCII, apoCIII, and total apoB (Table).

The proband is the youngest of 4 children; his 3 siblings were healthy, and all were heterozygous for the C65Y mutation. The parents, both heterozygotes, were first cousins (Figure 1D). Heterozygosity for the C65Y mutation was not associated with abnormal plasma lipid or apolipoprotein levels (Table and Figure 1C).

Figure 4. Assessing the amounts of GPIHBP1 at the surface of cells relative to the total amounts of GPIHBP1 in the cell with a goat antibody against the S-protein tag and a mouse monoclonal antibody against human GPIHBP1. A, Western blot analysis of GPIHBP1 at the cell surface. CHO pgsA-745 cells were electroporated with an empty vector or an expression vector encoding wild-type human GPIHBP1, GPIHBP1-C65Y, GPIHBP1-Q115P, or GPIHBP1-G56R. All constructs contained an amino-terminal S-protein tag. The next day, the cells were incubated for 2 hours at 4°C with a goat antiserum against the S-protein tag. After washing the cells 6 times in ice-cold PBS, the cell extracts were prepared for Western blotting with an IRdye680-conjugated donkey antibody against goat IgG and a mouse monoclonal antibody against human GPIHBP1. The mouse monoclonal antibody was detected with an IRdye800-conjugated donkey antibody against mouse IgG. B, Quantification of the Western blot. The signal corresponding to the goat anti-S-protein tag IgG was normalized to the signal for the monoclonal antibody against human GPIHBP1 and expressed relative to the ratio for wild-type GPIHBP1 (set at 100%).

GPIHBP1-C65Y Reaches the Cell Surface But Cannot Bind LPL
To determine whether the C65Y mutation interferes with the ability of the molecule to reach the cell surface, we generated an expression vector for GPIHBP1-C65Y and electroporated it into CHO pgsA-745 cells. In parallel, we performed an experiment with a GPIHBP1-C65A mutant. We used 2 previously characterized mutant GPIHBP1 proteins as controls: GPIHBP1-Q115P, which cannot bind LPL, and GPIHBP1-G56R, which binds LPL normally. By fluorescence microscopy, the amount of GPIHBP1-C65Y and GPIHBP1-C65A at the surface of nonpermeabilized cells seemed to be similar to that observed with cells expressing wild-type GPIHBP1 (Figure 2A). The amounts of GPIHBP1-Q115P and GPIHBP1-G56R proteins on the cell surface also appeared to be normal (Figure 2A). Fluorescence microscopy of permeabilized cells indicated that all of the cells expressed similar amounts of GPIHBP1 (Figure 2B).

We also assessed the amount of GPIHBP1 at the cell surface by 2 other techniques. First, we examined the amount of GPIHBP1 that could be released into the medium after incubating live cells with PIPLC. PIPLC released similar amounts of wild-type GPIHBP1 and GPIHBP1-C65Y, as judged by Western blotting (Figure 3), strongly suggesting that the C65Y mutation had little or no effect on the ability of the protein to reach the cell surface. Second, we tested the ability of an anti-S-protein tag antibody to bind to GPIHBP1 at the surface of live cells and compared that level of binding to the total amount of GPIHBP1 detectable in cells. Again, the amount of GPIHBP1-C65Y at the surface of cells was similar to the amount of GPIHBP1 at the surface of cells expressing wild-type GPIHBP1, GPIHBP1-Q115P, and GPIHBP1-G56R (Figure 4A). When the amount of GPIHBP1 at the cell surface was quantified and normalized to the total amount of GPIHBP1 in cells, we observed no differences...
GPIHBP1 Defects
Release of LPL by Heparin in Humans With GPIHBP1 Defects

We next assessed the ability of wild-type and mutant GPIHBP1 proteins to bind LPL. Human LPL bound avidly to cells expressing wild-type human GPIHBP1, but there was little if any LPL binding to cells expressing GPIHBP1-C65Y. Consistent with earlier studies, GPIHBP1-G56R bound LPL avidly but GPIHBP1-Q115P did not (Figure 5).

Figure 5. Assessing the binding of human V5-tagged LPL to GPIHBP1-C65Y. CHO pgsA-745 cells were electroporated with empty vector, an expression vector for wild-type human GPIHBP1, GPIHBP1-C65Y, GPIHBP1-Q115P, or GPIHBP1-G56R. Twenty-four hours later, the cells were incubated for 2 hours at 4°C with human V5-tagged LPL with or without heparin (500 U/mL). At the end of the incubation, the cells were washed 6 times with ice-cold PBS, and cell extracts were prepared for Western blotting. The amount of LPL bound to the cells was determined with a mouse monoclonal against the V5 tag; the levels of GPIHBP1 expression were assessed with a goat antibody against the S-protein tag. Actin was used as a loading control.

among any of the different GPIHBP1 constructs (Figure 4B). Thus, the mutant GPIHBP1 proteins were no different than wild-type GPIHBP1 in terms of reaching the cell surface.

Release of LPL by Heparin in Humans With GPIHBP1 Defects

When Gpihbp1−/− mice are given an intravenous bolus of heparin, LPL is released into the plasma, but with delayed kinetics. The plasma LPL levels in Gpihbp1−/− mice are low during the first few minutes after a heparin bolus, but after 15 minutes, the LPL levels are similar to those of wild-type mice. Whether the pattern of LPL release after heparin is perturbed in humans with GPIHBP1 mutations is unknown. To address this issue, we assessed the release of LPL into the plasma after an intravenous bolus of heparin in the GPIHBP1-C65Y homozygote (Figure 6A and 6B) and the GPIHBP1-Q115P homozygote (Figure 6C and 6D). Following heparin, LPL activity and mass levels were very low in each of multiple plasma samples obtained over an 18-minute time span (Figure 6A through 6D). The parents of the GPIHBP1-C65Y homozygote, both heterozygous carriers, had higher postheparin LPL mass and activity levels, as did unrelated control subjects (Figure 6A and 6B). Postheparin HL mass and activity were similar in the GPIHBP1-C65Y homozygote (Figure 6E and 6F), the heterozygous parents (Figure 6E and 6F), and the GPIHBP1-Q115P homozygote (Figure 6G and 6H).

In Gpihbp1−/− mice, the release of LPL into the plasma with heparin injections lowered plasma triglyceride levels. The same was the case in the human Q115P homozygote. During a 6-hour heparin infusion, plasma triglyceride concentrations gradually fell from 1780 to 534 mg/dL (20 to 6 mmol/L) (Figure 7A). A heparin infusion in normal subjects lowered triglyceride levels acutely, but then the triglyceride levels stabilized (Figure 7B). In normal subjects, LPL levels peaked very quickly following the bolus of heparin and remained high during the continuous heparin infusion (Figure 7C). In the Q115P homozygote, there was no sudden increase in LPL levels with the bolus of heparin, but LPL gradually appeared in the plasma during the heparin infusion.

Discussion

We identified a novel homozygous missense mutation in GPIHBP1, C65Y, in a young boy with chylomicronemia. Heterozygous carriers of the mutation were normolipidemic. A series of immunocytochemical, biochemical, and Western blot studies revealed that the cysteine-to-tyrosine substitution had little if any effect on the ability of GPIHBP1 to reach the cell surface. However, the mutation abolished the ability of the protein to bind LPL. Cells expressing wild-type GPIHBP1 or GPIHBP1-G56R bound LPL avidly, whereas cells expressing GPIHBP1-C65Y did not. The C65Y mutation represents the second example of a GPIHBP1 mutation that causes defective LPL binding. Beigneux et al previously showed that a young man with severe lifelong chylomicronemia was homozygous for another GPIHBP1 missense mutation (Q115P). Like GPIHBP1-C65Y, GPIHBP1-Q115P reached the cell surface but was unable to bind LPL. While we were preparing a revised version of this manuscript, Olivecrona et al described a family in which 3 siblings had severe chylomicronemia; all 3 had 2 mutant GPIHBP1 alleles. One of the mutant alleles contained a C65S mutation, whereas the other had a C68G mutation. These mutations abolished LPL binding but had little or no effect on GPIHBP1 trafficking to the plasma membrane. In light of these new findings, there are now 4 different missense mutations in the GPIHBP1 lymphocyte antigen 6 (Ly6) domain that have been associated with defective LPL binding. These findings strongly support the notion that the Ly6 domain is functionally important for LPL binding.

One of the important findings in this study is that the heterozygotes in the kindred had normal lipid levels. That finding strongly suggests that half-normal amounts of a functional GPIHBP1 are sufficient for normal lipolytic processing of triglyceride-rich lipoproteins.

GPIHBP1 is a member of a family of proteins containing a cysteine-rich Ly6 domain. The Ly6 domain contains either 8 or 10 cysteines arranged in a characteristic spacing pattern. The structures of several Ly6 proteins, for example CD59 (also known as protectin) and urokinase-type plasminogen activator receptor (UPAR), have been solved by NMR and x-ray crystallographic studies. These structures have demonstrated that each of the cysteines of the Ly6 domain is disulfide-bonded. In UPAR and CD59, the first cysteine of
Figure 6. The appearance of LPL and HL in the plasma after heparin. A and B, LPL in the plasma after an intravenous bolus of heparin in the subject homozygous for a C65Y mutation in GPIHBP1, his heterozygous parents, and 4 normolipidemic controls. LPL activity (A) and mass (B) in postheparin plasma were measured during an 18-minute sampling period after an intravenous injection of heparin (50 U/kg body weight). C and D, LPL in the plasma after an intravenous bolus of heparin in the subject homozygous for a Q115P mutation in GPIHBP1 and normolipidemic controls. LPL activity (C) and mass (D) in postheparin plasma was measured during an 18-minute sampling period after an intravenous injection of heparin (50 U/kg body weight). E and F, HL in the plasma after an intravenous bolus of heparin in the subject homozygous for a C65Y mutation in GPIHBP1, his heterozygous parents, and 4 normolipidemic controls. HL activity (E) and mass (F) in postheparin plasma were measured during an 18-minute sampling period after an intravenous injection of heparin (50 U/kg body weight). G and H, HL in the plasma after an intravenous bolus of heparin in the subject homozygous for a Q115P mutation in GPIHBP1 and normolipidemic controls. HL activity (G) and mass (H) in postheparin plasma was measured during an 18-minute sampling period after an intravenous injection of heparin (50 U/kg body weight). Proband is indicated with △; father, ○; mother, ▲; and controls, ■. For the controls (n=4), data are expressed as mean±SEM.
The fact that the elimination of a cysteine in the GPIHBP1 Ly6 domain would have important consequences for protein function is intriguing but not completely unexpected. Eliminating specific cysteines in the Ly6 domain of CD59 altered the ability of that protein to inhibit complement activation. Additionally, amino acid substitutions involving 2 different cysteines in the Ly6 domain of another Ly6 protein, secreted Ly6/UPAR-related protein 1, leads to a severe skin disease in humans (Mal de Meleda).27 Finally, the observation that a C65Y mutation interferes with protein function and actually leads to chylomicronemia is consistent with recent site-directed mutagenesis studies indicating that cysteine-to-alanine substitutions in GPIHBP1 interfere with the ability of the protein to bind LPL.16

A novel finding of this study is that the release of LPL into the plasma after an injection of heparin is abnormal in humans with GPIHBP1 mutations. When the C65Y and Q115P homozygotes were given a bolus of heparin, LPL mass and activity levels in the plasma remained very low over the entire 18 minutes of observation. It is intriguing that the LPL levels were lower in the 2 heterozygous carriers than in unrelated control subjects. However, the significance of this finding is uncertain, given that their lipid levels were entirely normal.

This observation of very low postheparin LPL levels in the 2 human homozygotes in this study was different from results in Gpihbp1−/− mice. Weinstein et al6 showed that LPL levels were abnormally low for several minutes following a bolus of heparin; however, by the 15-minute time point, the plasma LPL levels in Gpihbp1−/− were similar to those in control mice. In the human homozygotes, LPL levels were low initially and remained low. What accounts for the differences between mice and humans? We do not know the answer to this question, but one potential explanation relates to differences in the doses of heparin that were used. Most of the studies in mice involved a bolus injection of ~2000 IU/kg, whereas the humans were given 50 IU/kg. Had the human subjects been given a much higher dose of heparin, it is conceivable that we would have observed higher levels of LPL in their plasma. However, safety concerns related to the anticoagulant properties of heparin make it impossible to explore this possibility.

The origin of the LPL that is released into the plasma by heparin is not fully understood. Weinstein et al6 speculated that there are 2 pools of heparin-releasable LPL. One pool is located in the lumen of capillaries, bound to GPIHBP1, and another pool is located in the subendothelial spaces, perhaps bound to heparan sulfate proteoglycans.28 They suggested that the delayed release of LPL into the plasma of the Gpihbp1−/− mice was due to the absence of the luminal pool of LPL. In Gpihbp1−/− mice, the tissue stores of LPL were entirely normal. In wild-type mice, the tissue stores of LPL fell very little after an intravenous bolus of heparin, suggesting that most of the heparin-releasable LPL may be located in the subendothelial compartment. Whether GPIHBP1 deficiency in humans affects tissue LPL stores is unknown and needs to be investigated in the future.

In conclusion, we identified a homozygous missense mutation in GPIHBP1 in a patient with severe chylomicronemia.

Figure 7. LPL mass and triglycerides in the plasma during a continuous infusion of heparin in the Q115P homozygote and controls. We analyzed LPL mass and triglyceride levels in multiple samples taken every 30 minutes. A, Plasma triglycerides in the Q115P homozygote. B, Plasma triglycerides in normolipidemic controls. For the experiments shown in A and B, an inhibitor of lipolysis, tetrahydrolipstatin, was added to the blood after samples were collected. Thus, the fall in plasma triglycerides observed in A could not be ascribed to continuing lipolysis occurring in the tube ex vivo. C, Plasma LPL mass measurements in the normolipidemic controls and the Q115 P homozygote. Proband is indicated with ▲ and controls, ■. For the controls (n=7), data are expressed as mean±SEM.

the Ly6 domain 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. The 5 disulfide bonds of the Ly6 domain create a 3-fingered structural motif.22 Currently, the structure of GPIHBP1 is not known, but we have generated a likely model of the GPIHBP1 Ly6 domain based on the known structure of CD59 (Figure 8).

Precisely how the C65Y substitution alters the structure of GPIHBP1 is not known. However, this mutation eliminates the first cysteine of the Ly6 domain and therefore would interfere with the disulfide bond between the first and fifth cysteine (C89) of the Ly6 domain. We suspect that the elimination of the C65-C89 disulfide bond disrupts the 3-fingered structure of the GPIHBP1 Ly6 domain.
Cell culture studies revealed that the mutant protein reached the cell surface but could not bind LPL. An important and novel finding of the current studies is that GPIHBP1 defects in humans are associated with very low levels of LPL in postheparin plasma. Together with the Q115P studies reported earlier and the very recent mutagenesis studies on GPIHBP1, our current studies provide strong evidence that GPIHBP1 is important for the lipolytic processing of triglyceride-rich lipoproteins in humans.

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Disclosures
None.

References
CLINICAL PERSPECTIVE

Glycosylphosphatidylinositol-anchored high-density lipoprotein-binding protein 1 (GPIHBP1) is an endothelial protein of the lymphocyte antigen 6 family. GPIHBP1 binds lipoprotein lipase (LPL), and recent experiments with genetically modified mice have revealed that GPIHBP1 plays an essential role in the lipolytic processing of triglyceride-rich lipoproteins. The importance of GPIHBP1 in humans has been far less certain, and the present investigation addresses that issue. Previously, there was only one link between GPIHBP1 and hypertriglyceridemia (the identification of a homozygous Q115P mutation in a young man with chylomicronemia). In the current investigation, we identify a homozygous C65Y mutation in the lymphocyte antigen 6 domain of GPIHBP1 in a young boy with severe chylomicronemia and demonstrate that this mutation abolishes the ability of the protein to bind LPL. From the basic science perspective, these studies are important because they show that the lymphocyte antigen 6 domain of GPIHBP1 is critical for the binding of LPL. Our investigation is also important from the standpoint of clinical lipidology. When faced with a patient with severe hypertriglyceridemia, one of the questions is, “What are the LPL levels?” When it comes to the hypertriglyceridemia caused by GPIHBP1 deficiency, the answer to that question was unknown. In this investigation, we show in 2 subjects with GPIHBP1 defects that the postheparin levels of circulating LPL are extremely low and that LPL enters plasma with delayed kinetics.
Chylomicronemia With Low Postheparin Lipoprotein Lipase Levels in the Setting of GPIHBPI Defects
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