Chylomicronemia With a Mutant GPIHBP1 (Q115P) That Cannot Bind Lipoprotein Lipase

Anne P. Beigneux, Remco Franssen, André Bensadoun, Peter Gin, Kristan Melford, Jorge Peter, Rosemary L. Walzem, Michael M. Weinstein, Brandon S.J. Davies, Jan A. Kuivenhoven, John J.P. Kastelein, Loren G. Fong, Geesje M. Dallinga-Thie, Stephen G. Young

Objective—GPIHBP1 is an endothelial cell protein that binds lipoprotein lipase (LPL) and chylomicrons. Because GPIHBP1 deficiency causes chylomicronemia in mice, we sought to determine whether some cases of chylomicronemia in humans could be attributable to defective GPIHBP1 proteins.

Methods and Results—Patients with severe hypertriglyceridemia (n=60, with plasma triglycerides above the 95th percentile for age and gender) were screened for mutations in GPIHBP1. A homozygous GPIHBP1 mutation (c.344A>C) that changed a highly conserved glutamine at residue 115 to a proline (p.Q115P) was identified in a 33-year-old male with lifelong chylomicronemia. The patient had failure-to-thrive as a child but had no history of pancreatitis. He had no mutations in LPL, APOA5, or APOC2. The Q115P substitution did not affect the ability of GPIHBP1 to reach the cell surface. However, unlike wild-type GPIHBP1, GPIHBP1-Q115P lacked the ability to bind LPL or chylomicrons (d < 1.006 g/mL lipoproteins from Gpihbp1−/− mice). Mouse GPIHBP1 with the corresponding mutation (Q114P) also could not bind LPL.

Conclusions—A homozygous missense mutation in GPIHBP1 (Q115P) was identified in a patient with chylomicronemia. The mutation eliminated the ability of GPIHBP1 to bind LPL and chylomicrons, strongly suggesting that it caused the patient’s chylomicronemia. (Arterioscler Thromb Vasc Biol. 2009;29:956-962.)

Key Words: lipoprotein • lipase • human • chylomicronemia • hypertriglyceridemia • GPIHBP1

Chylomicronemia can be caused by a deficiency of lipoprotein lipase (LPL) or apolipoprotein (apo) CII. However, many cases of chylomicronemia in humans are unexplained. In 2007, Beigneux et al identified a new potential cause of chylomicronemia, a deficiency of glycosylphosphatidylinositol-anchored high-density lipoprotein–binding protein 1 (GPIHBP1).

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Mice lacking GPIHBP1 manifest severe chylomicronemia, even on a low-fat chow diet, with plasma triglycerides >2000 mg/dL. GPIHBP1 is found on the luminal surface of capillaries in heart, skeletal muscle, and adipose tissue, where the lipolytic processing of triglyceride-rich lipoproteins occurs. Transfection of a GPIHBP1 expression vector into CHO cells confers the ability to bind LPL, chylomicrons, as well as apo-AV–phospholipid disks. The ability of GPIHBP1-expressing cells to bind LPL and chylomicrons suggested that GPIHBP1 might function as a platform for lipolysis on endothelial cells.

Two structural features of GPIHBP1 are important in the binding of LPL and chylomicrons. The first is an amino-terminal acidic domain, approximately 25 amino acids in length. Mutant GPIHBP1 proteins lacking all or part of the acidic domain are unable to bind LPL and chylomicrons. The second is a Lymphocyte antigen 6 (Ly6) domain. Ly6 motifs, which contain either 8 or 10 cysteines with a characteristic spacing pattern, are found in a number of GPI-anchored proteins, for example CD59 and the urokinase-type plasminogen activator receptor (UPAR). When the Ly6 domain of GPIHBP1 is replaced with the Ly6 domain from CD59, the chimeric protein reaches the cell surface but cannot bind LPL, even though the acidic domain of GPIHBP1 is intact.

All mammalian GPIHBP1 proteins share the acidic domain and the Ly6 domain (with 10 conserved cysteines). The highest level of amino acid conservation lies within a portion of the Ly6 domain (residues 101 to 121 in human GPIHBP1, which contains the 6th and 7th cysteines of the Ly6 motif). The finding of chylomicronemia in Gpihbp1−/− mice raised the question of whether some cases of chylomicrone-
mia in humans might be caused by GPIHBP1 mutations. Wang and Hegel spread GPIHBP1 coding sequences in 160 patients with chylomicronemia and identified only 1 variant, a homozygous G56R mutation in 2 siblings. Residue 56 is located in a linker segment between the acidic domain and the Lys6 domain. Recently, Qin et al examined the functional properties of the G56R mutant in CHO cells and could not find any deficit in the ability of the mutant protein to reach the cell surface or its ability to bind LPL, chylomicrons, or apoAV phospholipids disks. Those findings raised doubts about whether the G56R mutation truly caused the hyperlipidemia.

In this study, we sequenced the coding regions of GPIHBP1 in 60 unrelated adults with unexplained chylomicronemia. One patient, a 33-year-old male, was homozygous for a missense mutation (Q115P) within the most highly conserved portion of the Ly6 domain. Cell culture studies revealed that the mutant GPIHBP1 reached the cell surface normally but could not bind LPL or chylomicrons.

**Methods**

**Subjects**

Patients with chylomicronemia (n = 60; plasma triglycerides, 446 ± 3366 mg/dL; postheparin plasma LPL mass and activity, 79.5 ± 48.7 ng/mL and 59.9 ± 63.9 ng/mL, respectively) were identified within the Lipid Clinic of the Academic Medical Center Amsterdam (AMC). After excluding mutations in LPL, APOA5, and APOC2, the coding regions of GPIHBP1 (NM_178172) were amplified and sequenced. A homozygous missense mutation in GPIHBP1 (c.344A>C; p.Q115P) was identified in a 33-year-old male with severe lifelong chylomicronemia.

Three normolipidemic age-matched men and an LPL-deficient patient (a compound heterozygote for V69L and G188E mutations) were used as controls. Studies were approved by the Committee on Human Research at AMC and UCLA.

**Genomic DNA Analysis**

Genomic DNA was prepared from blood leukocytes. The 4 exons of GPIHBP1, along with ∼50 bp of introns on either side, were amplified with primers described in supplemental Table I (available online at http://atvb.ahajournals.org). The exons of LPL, APOA5, and APOC2 were amplified with the primers listed in supplemental Table II. An M13 tail was added to each primer (forward: 5'-GTTGTAAAAACGAGGCACCCT-3'; reverse: 5'-CACAGGAAA-CAGCTATGACC-3') to facilitate DNA sequencing.

**Biochemical Measurements**

Total plasma cholesterol, triglycerides, high-density lipoprotein (HDL) cholesterol, and low-density lipoprotein (LDL) cholesterol levels were determined with commercial kits (Wako). Plasma apoB, apoC-II, and apoC-III 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 (FPLC); online triglyceride measurements were obtained with a commercial assay (Biomenerex). Blood was obtained before and 18 minutes after an intravenous injection of heparin (50 IU/kg body weight). LPL and hepatic lipase (HL) activity levels were assessed as previously described. Plasma LPL mass levels were measured with an ELISA (Daiichi); HL mass levels were also measured with an ELISA.

**GPIHBP1 Constructs and Cell Transfections**

Untagged and S-protein–tagged versions of mouse and human GPIHBP1 have been described previously. A mouse “D.E(38-48)A” GPIHBP1 mutant (in which the aspartate and glutamates between residues 38 and 48 were changed to alanine) was described previously. Human GPIHBP1-Q115P and mouse GPIHBP1-Q114P expression vectors were generated by site-directed mutagenesis with the QuickChange kit (Stratagene). Transient transfections of CHO pgsA-745 cells (a mutant CHO cell line with deficient sulfation of heparan sulfate proteoglycans) were performed with Lipofectamine 2000 (Invitrogen) or by electroporation with the Nucleofector II apparatus (Lonza).

To determine whether GPIHBP1 proteins reached the cell surface, we assessed the release of GPIHBP1 into the cell culture medium after treating cells with a phosphatidylinositol-specific phospholipase C (PIPLC, 1 U/mL for 1 hour at 37°C). GPIHBP1 in the medium and cell extracts was assessed by Western blotting.

**Binding of Human LPL to GPIHBP1-Expressing CHO pgsA-745 Cells**

In some experiments, CHO pgsA-745 cells were cotransfected with expression vectors for a V5-tagged human LPL (gift from Dr Mark Doolittle, University of California, Los Angeles) and GPIHBP1. After 24 hours, the cells were incubated for 15 minutes at 37°C in the absence or presence of heparin (1 U/mL). The medium was harvested and cell extracts were collected in RIPA buffer containing complete mini EDTA-free protease inhibitors (Roche). LPL in the medium and GPIHBP1 and LPL in cell extracts were assessed by Western blotting.

In other experiments, CHO pgsA-745 cells were electroporated with GPIHBP1 (or empty vector) and then incubated for 2 hours at 4°C with 200 μL of conditioned medium from either nontransfected CHO cells or CHO cells that had been stably transfected with a V5-tagged human LPL expression vector. In a given experiment, each well was incubated with exactly the same amount of LPL. In some wells, heparin (500 U/mL) was added to the medium. At the end of the incubation period, cells were washed 6 times with ice-cold PBS containing 1.0 mmol/L MgCl2 and 1.0 mmol/L CaCl2. Levels of GPIHBP1 and LPL in cell extracts were assessed by Western blotting.

The binding of avian LPL to mouse or human GPIHBP1 was performed as described. Levels of mouse GPIHBP1 in cell lysates were assessed with a sandwich ELISA using immunopurified rabbit antibodies against GPIHBP1; levels of human GPIHBP1 in cell lysates were assessed by western blotting.

**Western Blot Analyses**

Proteins were size-fractionated on 4% to 12% Bis-Tris SDS-polyacrylamide gels, and Western blotting was performed as described. The antibody dilutions were 1:250 for a mouse monoclonal antibody against human apoB-1; 1:2000 for an IRdye800-conjugated goat antimouse IgG (Li-Cor); 1:500 for a goat polyclonal antibody against human apoA5; 1:200 for a mouse monoclonal antibody against human apoC2, and 1:500 for an IRdye680-conjugated donkey antimouse IgG (Li-Cor); 1:500 for an IRdye800-conjugated donkey antirabbit IgG (Li-Cor); 1:6000 for an IRdye800-conjugated donkey anti-rabbit IgG (Li-Cor); and 1:500 for an IRdye800-conjugated donkey antihuman IgG (Li-Cor). Antibody binding was detected with an Odyssey infrared scanner (Li-Cor).

**Binding of Dil-Labeled Chylomicrons to Transfected Cells**

The d < 1.006 g/mL lipoproteins from Gpihbp1−/− mice (“chylomicrons”) were labeled with Dil (1,1′dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate), and the binding of the Dil-labeled chylomicrons to human GPIHBP1-expressing CHO pgsA-745 cells was assessed by fluorescence microscopy.

**Results**

**Identification of a Missense Mutation in GPIHBP1 in a Patient With Chylomicronemia**

The GPIHBP1 coding sequences were examined in 60 patients with chylomicronemia; none of these patients had
coding sequence variations in \textit{LPL}, \textit{APOC2}, or \textit{APOA5}. One noteworthy \textit{GPIHBP1} mutation was identified. An A to C transversion in exon 4 of \textit{GPIHBP1} (c.344A$\rightarrow$C, resulting in a Q115P substitution) was identified in a 33-year-old male with chylomicronemia. The mutation was documented by bidirectional DNA sequencing (Figure 1A) and confirmed by digesting DNA samples with \textit{AciI} (Figure 1B). The patient was born in Columbia and adopted by a Dutch family. He exhibited hepatosplenomegaly and failure to thrive as a child and was diagnosed with type I hyperlipoproteinemia at the age of 7. The patient had a normal BMI (24.4) and normal glucose levels. Fasting chylomicronemia was documented on multiple occasions. The hyperlipidemia was partially responsive to diet; fasting plasma triglyceride levels fell from as high as 3366 mg/dL to as low as 744 mg/dL when the patient adhered to a fat-free diet (Table). The patient had lipemia retinalis but had no history of eruptive xanthomas or pancreatitis.

The plasma of the proband contained increased amounts of apo-B48, as judged by Western blots (Figure 1C and 1D) and an ELISA (Table). Size-fractionation of the patient’s plasma revealed that most of the triglycerides were in large lipoproteins (ie, the “VLDL” peak; Figure 1E). The HDL-cholesterol levels were low (Table). The diameters of the patient’s triglyceride-rich lipoproteins were far larger than those of normolipidemic controls (Figure 1F). Postheparin LPL mass levels were 36 ng/mL (=10% of normal), whereas HDL mass levels were normal (273 ng/mL; Table). LPL activity and mass levels were comparably low, indicating that the specific activity of the LPL was normal (Table). Apo-CII levels in the plasma were elevated, compared with normolipidemic controls (18.5 mg/dL versus 7.2$\pm$2.1 mg/dL, respectively; Table). Apo-CIII levels were also elevated (Table).

**GPIHBP1-Q115P Reaches the Cell Surface Normally But Cannot Bind LPL**

To determine whether the Q115P mutation blocks the ability of \textit{GPIHBP1} to reach the cell surface, wild-type and mutant (Q115P) \textit{GPIHBP1} constructs were transfected into \textit{CHO} cells. We then assessed the release of \textit{GPIHBP1} into the culture medium after treating the cells with a phosphatidylinositol-specific phospholipase C (PIPLC). Wild-type \textit{GPIHBP1} and \textit{GPIHBP1}-Q115P yielded similar levels of expression in cells, and similar amounts of \textit{GPIHBP1} were released into the cell culture medium by PIPLC, showing that the Q115P mutation did not interfere with the ability of \textit{GPIHBP1} to reach the cell surface (Figure 2A). To further explore this issue, we used immunofluorescence microscopy along with an acidic domain antiserum to detect wild-type and mutant versions of \textit{GPIHBP1} on the surface of nonpermeabilized cells. For these studies, we examined the expression of wild-type mouse \textit{GPIHBP1}, a mutant mouse \textit{GPIHBP1} with the analogous mutation (Q114P in the mouse sequence), and a mutant \textit{GPIHBP1} in which the acidic domain had been mutated (as a negative control). In side-by-side experiments, the amounts of wild-type \textit{GPIHBP1} and \textit{GPIHBP1}-Q115P...
Q114P on the surface of cells were comparable (Figure 2B). When similar experiments were carried out with human GPIHB1 constructs, the amounts of wild-type and mutant (Q115P) GPIHB1 on the surface of cells were comparable (Figure 2C).

The ability of human GPIHB1-Q115P to bind LPL was assessed with a Western blot assay. CHO pgsA-745 cells were transfected with a human LPL expression vector, alone or in combination with expression vectors for wild-type or mutant versions of human GPIHB1. Large amounts of wild-type LPL appeared in the culture medium of cells that did not express GPIHB1 (Figure 3A). When cells expressed wild-type GPIHB1, little LPL was detected in the culture medium (because it was bound to GPIHB1 on the cell surface). However, the GPIHB1-bound LPL could be released into the medium with heparin (Figure 3A). When the same experiment was performed with the GPIHB1-Q115P mutant, large amounts of LPL were found in the medium, and little additional LPL was released by heparin, suggesting that GPIHB1-Q115P binds LPL poorly (Figure 3A). To further explore this issue, we tested the ability of mouse GPIHB1-Q114P to bind LPL. When CHO pgsA-745 cells were cotransfected with human LPL and wild-type mouse GPIHB1, little LPL was detected in the cell culture medium (because it was bound to GPIHB1 on the cell surface), but the LPL could be released into the medium with heparin (Figure 3B). When the same experiment was performed with the GPIHB1-Q114P mutant, significant amounts of LPL were found in the medium, and no additional LPL was released by heparin, indicating that mouse GPIHB1-Q114P bound LPL poorly (Figure 3B). In this same experiment, human GPIHB1-Q115P bound LPL poorly (Figure 3B).

We also assessed the binding of LPL to wild-type and mutant GPIHB1 with direct binding assays. In these studies, wild-type and mutant versions of GPIHB1 were transiently expressed into CHO pgsA-745 cells. Cells were incubated with a V5-tagged human LPL for 2 hours at 4°C. After washing the cells, the amount of LPL bound to the cells was assessed by Western blotting. Cells expressing wild-type human or mouse GPIHB1 bound LPL, but cells expressing mouse GPIHB1-Q114P or human GPIHB1-Q115P did not bind LPL (Figure 4A). In similar experiments, we incubated GPIHB1-expressing cells with purified avian LPL, and the amount of LPL bound to cells was assessed with a monoclonal antibody–based ELISA. Cells expressing wild-type human GPIHB1 bound 39.8-fold more LPL than cells expressing GPIHB1-Q115P (Figure 4B), and cells expressing wild-type mouse GPIHB1 bound 20.2-fold more LPL than cells expressing GPIHB1-Q114P (Figure 4C).

<p>| Table. Plasma Lipid and Apolipoprotein Concentrations in the Proband |
|-------------------------|------------------|------------------|</p>
<table>
<thead>
<tr>
<th></th>
<th>Q115P (n=1)</th>
<th>LPL-Deficient (n=1)</th>
<th>Controls (n=3)</th>
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<tbody>
<tr>
<td>TG, mg/dl</td>
<td>744</td>
<td>779</td>
<td>88.6±26.6</td>
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<tr>
<td>TC, mg/dl</td>
<td>120</td>
<td>108</td>
<td>178±34.8</td>
</tr>
<tr>
<td>LDLc, mg/dl</td>
<td>81.2</td>
<td>58.0</td>
<td>120±19.3</td>
</tr>
<tr>
<td>HDLc, mg/dl</td>
<td>8.9</td>
<td>5.5</td>
<td>42.5±7.7</td>
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<tr>
<td>Apo-B, µg/ml</td>
<td>98</td>
<td>103</td>
<td>117±19</td>
</tr>
<tr>
<td>Apo-B48, µg/ml</td>
<td>35.4</td>
<td>30.5</td>
<td>5.0±2.0</td>
</tr>
<tr>
<td>Apo-CII, mg/dl</td>
<td>18.5</td>
<td>14.6</td>
<td>1.5±1.3</td>
</tr>
<tr>
<td>Apo-CIII, mg/dl</td>
<td>17.4</td>
<td>18.5</td>
<td>7.2±2.1</td>
</tr>
<tr>
<td>LPL activity, mU/ml</td>
<td>40</td>
<td>19</td>
<td>275±122</td>
</tr>
<tr>
<td>LPL mass, ng/ml</td>
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<td>273</td>
<td>421±55</td>
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<td>HDL mass, mg/dl</td>
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<td>342</td>
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<td>342</td>
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</table>

Plasma concentrations of triglycerides (TG), total cholesterol (TC), low-density lipoprotein cholesterol (LDLc), high-density lipoprotein cholesterol (HDLc), and of apolipoproteins (apo) in 3 normolipidemic controls, the proband (Q115P), and a patient with a mutation in the catalytic domain of LPL (LPL-deficient). LPL and HL mass and activity were performed on plasma collected 18 minutes after an intravenous heparin injection. Data are presented as mean±SD.
GPIHBP1-Q115P Binds Chylomicrons Poorly

The expression of GPIHBP1 in CHO pgsA-745 cells confers on cells the ability to bind chylomicrons (d=1.006 g/mL lipoproteins from Gpihbp1−/− mice).2 To assess the impact of the Q115P mutation on chylomicron binding, we compared the binding of DiI-labeled chylomicrons to CHO pgsA-745 cells expressing wild-type GPIHBP1 or GPIHBP1-Q115P. Cells expressing wild-type GPIHBP1 bound chylomicrons avidly, whereas cells expressing GPIHBP1-Q115P did not (Figure 5).

Discussion

The importance of GPIHBP1 for lipolysis in mice is undeniable; Gpihbp1−/− mice have plasma triglyceride levels of ≈3000 to 5000 mg/dL, similar to mice lacking LPL.15 To determine whether GPIHBP1 mutations might account for some cases of chylomicronemia in humans, we sequenced the coding sequences of GPIHBP1 in 60 unrelated patients with unexplained chylomicronemia. We identified one noteworthy mutation, a homozygous Q115P mutation in a 33-year-old male with Type I hyperlipoproteinemia since childhood. The amino acid substitution was located in the most highly conserved segment of the Ly6 motif of GPIHBP1.6 The Q115P mutation in human GPIHBP1 (and the Q114P mutation in mouse GPIHBP1) eliminated the ability of the molecule to bind human or avian LPL. GPIHBP1-Q115P also lacked the ability to bind chylomicrons. These results firmly establish that GPIHBP1-Q115P is dysfunctional in cell culture assays. Thus, our studies identified striking functional abnormalities in GPIHBP1 in a patient with severe lifelong chylomicronemia. It seems overwhelmingly likely that the Q115P mutation is responsible for the patient’s chylomicronemia and that we have identified the first clinically significant GPIHBP1 mutation in humans. Our findings are important because they indicate that GPIHBP1 is important for lipolysis in humans (as it is in the mouse).
Figure 5. Decreased binding of Dil-labeled chylomicrons to cells expressing GPIHBP1-Q115P. CHO pgsA-745 cells were transfected with wild-type or mutant (Q115P) S-protein–tagged human GPIHBP1 expression vectors. GPIHBP1 on the surface of cells was detected by immunofluorescence microscopy with an antibody against the S-protein tag (green). Chylomicron binding was detected with Dil-fluorescence (red). Cell nuclei were visualized with DAPI (blue).
Our studies identified only 1 mutation in 60 patients with chylomicronemia, reinforcing the view that mutations in GPIHBP1 are an uncommon cause of chylomicronemia in humans. The fact that the Q115P proband had been adopted meant that no family studies were possible. An analysis of the pedigree would have been interesting, mainly to document the impact of heterozygous GPIHBP1 mutations in humans. In mice, heterozygosity for Gpihbp1 deficiency has no effect on plasma lipid levels.

The level of LPL in the postheparin plasma of the Q115P patient was low. Levels of LPL were also low in Gpihbp1−/− mice after an intraperitoneal injection of heparin. After an intravenous injection of heparin, LPL levels in Gpihbp1−/− mice were low at early time points but then increased to near-normal levels. Further study of LPL levels would be interesting but will require the identification of additional GPIHBP1-deficient patients.

Missense mutations in the LDL receptor often lead to retention of the protein in the ER, preventing it from reaching the cell surface. We considered the possibility that the Q115P mutation might interfere with the trafficking of GPIHBP1 to the cell surface, but this did not appear to be the case. Wild-type and mutant GPIHBP1 were expressed at comparable levels at the surface of cells, as judged by immunocytochemistry and by PIPLC release.

The fact that a structural alteration in the Ly6 domain would interfere with the binding of ligands is not particularly surprising. In the case of other GPI-anchored Ly6 proteins, for example UPAR or CD59, the Ly6 motif represents the ligand-binding domain, so it is quite plausible that the Q115P mutation might interfere with the trafficking of GPIHBP1. That chimeric protein reached the cell surface.17 We considered the possibility that the Q115P substitution might interfere with the trafficking of GPIHBP1 to the cell surface, but this did not appear to be the case. Wild-type and mutant GPIHBP1 were expressed at comparable levels at the surface of cells, as judged by immunocytochemistry and by PIPLC release.

In summary, we identified a homozygous missense mutation in GPIHBP1 (p.Q115P) in a patient with lifelong chylomicronemia. This amino acid substitution blocked the ability of GPIHBP1 to bind LPL and chylomicrons, suggesting that it was responsible for the patient’s hyperlipidemia. These studies also show that GPIHBP1 is crucial for the lipolytic processing of lipoproteins in humans.

Acknowledgments

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Disclosures

None.

References


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## SUPPLEMENT MATERIAL

Supplemental Table I. Amplification and sequencing primers for *GPIHBP1*.

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<th>Region</th>
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| Promoter | F: 5′-GAGGGGTGTCAGGCATGG-3′  
             R: 5′-AGAGGGCAAGCAGGACAG-3′ | 400 |
| Promoter | F: 5′-GACCCGAGGTGATAGTGAGG-3′  
             R: 5′-AGTCATTGTTGGTGCTCTTG-3′ | 399 |
| Promoter | F: 5′-AGAGCCCTCGACTCTGCACTC-3′  
             R: 5′-AACACAGTCAATGCCCCTCAAG-3′ | 375 |
| Exon 1   | F: 5′-CTTCATCCCATCACTACGAGCAG-3′  
             R: 5′-CTCTTCTTCTCTCCTAAGCCCCTG-3′ | 226 |
| Exon 2   | F: 5′-ATGCTTGCCCAGAGCAGGCTGTC-3′  
             R: 5′-GCCTGCTGGCTCTCCATCACAC-3′ | 282 |
| Exon 3   | F: 5′-CATCTGACAGTGTTGCTGGT-3′  
             R: 5′-AGGTGGCTCTCGAGGGGCTC-3′ | 330 |
| Exon 4   | F: 5′-AACACCGGTAAAGTTGGGCGTG-3′  
             R: 5′-CAAATCCATTCTCTCAAGCTGG-3′ | 504 |

F, forward primer; R, reverse primer.
Supplemental Table II. Amplification and sequencing primers for *LPL*, *APOA5*, and *APOC2*.

<table>
<thead>
<tr>
<th>Region</th>
<th>Primer sequence</th>
<th>Product (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>LPL</strong></td>
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<td></td>
</tr>
<tr>
<td>Promoter F: 5′-GAGGGAGGACTGCAAGTGAC-3′</td>
<td>443</td>
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</tr>
<tr>
<td>R: 5′-AGCTTTCCCTTGAGGAGGAG-3′</td>
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<tr>
<td>Promoter F: 5′-ATTTGACCTCGATGTTCTGC-3′</td>
<td>400</td>
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</tr>
<tr>
<td>R: 5′-ACAGGTTCACATCGATTCAG-3′</td>
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<tr>
<td>Exon 1  F: 5′-GTAGAGTGGAACCCCTTAAGCTAAGCG-3′</td>
<td>548</td>
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<tr>
<td>R: 5′-ACGCCCGGTCTGCAAGTGAGGG-3′</td>
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<tr>
<td>Exon 2  F: 5′-AACCCCTCCATTAACCTATATCC-3′</td>
<td>241</td>
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<tr>
<td>R: 5′-CACCAACCCAATCCACTTCCC-3′</td>
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<tr>
<td>Exon 3  F: 5′-GAACAGGCCGTTTTCTGGCTCCAGTC-3′</td>
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<tr>
<td>R: 5′-GCTAGGTGGGTATTTAAGAAAGCTTGTG-3′</td>
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<tr>
<td>Exon 4  F: 5′-GAATTAGTTTTTCAGTATTTCTATATTTTG-3′</td>
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<tr>
<td>R: 5′-CTCTCAAGATGACAGTCTTTTCACC-3′</td>
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<tr>
<td>Exon 5  F: 5′-GCAGTGACATGCGAATGTCAC-3′</td>
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<tr>
<td>R: 5′-GGACATTGGGTCAATAAGGG-3′</td>
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<tr>
<td>Exon 6  F: 5′-CCACATCTCACCATTGGACATGCG-3′</td>
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<tr>
<td>R: 5′-GCAGTGACATGGAATAGGACATGCG-3′</td>
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<tr>
<td>Exon 7  F: 5′-GAGTTCATGTGTGACTCTCCCGG-3′</td>
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<tr>
<td>R: 5′-CAGGAGAGGGACTGTTGCATGTG-3′</td>
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</tr>
<tr>
<td>Exon 8  F: 5′-AAGTGGGGGCGAGAGAGCTGATC-3′</td>
<td>291</td>
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<tr>
<td>R: 5′-CATCAGGTGGGGGTCTAAAGTGAGG-3′</td>
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</tbody>
</table>
Exon 9  
F: 5′-GATTCTGATGTCGCTGGAGTGAGACAG-3’  358  
R: 5′-GCTGGTATGGGTTGAAGAGAATGC-3’  

Exon 10  
F: 5′-CACATCTCTCCCTGGGTATTATCTCAC-3’  562  
R: 5′-GTAAGTTTGAAGGCCTCAGTC-3’

**APOA5**

Exons 1–2  
F: 5′-TAACAGGATTCGGCGAGG-3’  484  
R: 5′-ACAGAGGTTGAGGCGAGCAGA-3’  

Exon 3  
F: 5′-GATGACTTGGGAGACAAAGGA-3’  394  
R: 5′-ACTGGGCTTGGTCTCC-3’

**APOC2**

Exon 1  
F: 5′-GCTGTTGCCAAGTCCATGC-3’  379  
R: 5′-GGGGGAGAGTGTGTCAGGAG-3’  

Exon 2  
F: 5′-CTGCCCTCTCCTCTTC-3’  302  
R: 5′-TCTGGTGCTGATGCGT-3’

Exon 3  
F: 5′-CATACCTGCCCAGCTAGAT-3’  538  
R: 5′-TCAGGCTAGTGTTGGGAGGA-3’

F, forward primer; R, reverse primer.