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Original Article

# An enzyme-linked immunosorbent assay for measuring GPIHBP1 levels in human plasma or serum

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Urokinase-type plasminogen activator receptor;
Lymphocyte antigen 6

**BACKGROUND:** Glycosylphosphatidylinositol-anchored high-density lipoprotein–binding protein 1 (GPIHBP1), a glycosylphosphatidylinositol (GPI)-anchored protein of capillary endothelial cells, transports lipoprotein lipase to the capillary lumen and is essential for the lipolytic processing of triglyceride-rich lipoproteins.

**OBJECTIVE:** Because some GPI-anchored proteins have been detected in plasma, we tested whether GPIHBP1 is present in human blood and whether *GPIHBP1* deficiency or a history of cardiovascular disease affected GPIHBP1 circulating levels.

**METHODS:** We developed 2 monoclonal antibodies against GPIHBP1 and used the antibodies to establish a sandwich enzyme-linked immunosorbent assay (ELISA) to measure GPIHBP1 levels in human blood.

**RESULTS:** The GPIHBP1 ELISA was linear in the 8 to 500 pg/mL range and allowed the quantification of GPIHBP1 in serum and in pre- and post-heparin plasma (including lipemic samples). GPIHBP1 was undetectable in the plasma of subjects with null mutations in *GPIHBP1*. Serum GPIHBP1 median levels were 849 pg/mL (range: 740–1014) in healthy volunteers ($n = 28$) and 1087 pg/mL (range: 877–1371) in patients with a history of cardiovascular or metabolic disease ($n = 415$). There was an extremely small inverse

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correlation between GPIHBP1 and triglyceride levels ($r = 0.109$; $P < .0275$). GPIHBP1 levels tended to be slightly higher in patients who had a major cardiovascular event after revascularization.

**CONCLUSION:** We developed an ELISA for quantifying GPIHBP1 in human blood. This assay will be useful to identify patients with *GPIHBP1* deficiency and patients with GPIHBP1 autoantibodies. The potential of plasma GPIHBP1 as a biomarker for metabolic or cardiovascular disease is yet questionable but needs additional testing.

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## Introduction

Lipoprotein lipase (LPL), a triglyceride hydrolase secreted by myocytes and adipocytes, is crucial for the lipolytic processing of triglyceride-rich lipoproteins along capillaries.[1–3] The mechanism by which LPL reaches its site of action inside blood vessels was mysterious for decades, but the mystery was ultimately solved by Beigneux et al. and Davies et al.[4,5] They showed that glycosylphosphatidylinositol-anchored high-density lipoprotein–binding protein 1 (GPIHBP1), a GPI-anchored protein of capillary endothelial cells, binds LPL in the subendothelial spaces and shuttles it across endothelial cells to its site of action in the capillary lumen. A deficiency in GPIHBP1 causes severe hypertriglyceridemia (chylomicronemia).[6–11]

GPIHBP1 is a member of the Ly6/uPAR (“LU”) protein superfamily. It contains an amino-terminal acidic domain[12] and an ∼80 amino acid “Ly6 domain.” The Ly6 domain contains 10 cysteines, all in a characteristic spacing pattern and all disulfide bonded, so as to create a 3-finger fold.[13] Biophysical studies revealed that the Ly6 domain is largely responsible for high-affinity LPL binding to GPIHBP1, whereas the acidic domain has only an accessory role in LPL binding[14] and is more relevant to the stability of LPL activity.[14,15] Consistent with these findings, a monoclonal antibody (mAb) against GPIHBP1’s Ly6 domain (RE3) was shown to block LPL binding to GPIHBP1, whereas an mAb against GPIHBP1’s acidic domain (RF4) did not.[16]

GPI-anchored proteins can be released from the plasma membrane by several mechanisms, for example, the release of vesicles, cleavage of the polypeptide, or cleavage of the GPI anchor by GPIases such as GPI-specific phospholipase D.[17,18] As a result, some GPI-anchored proteins can be detected in the plasma. One example is urokinase-type plasminogen activator receptor (uPAR).[19] Interestingly, the levels of uPAR in the plasma have been shown to be elevated in certain cancers, and increased uPAR levels are associated with a poor prognosis[20]; noninvasive PET-imaging reveals increased uPAR expression in the solid tumors and their metastases.[21]

We set out to determine if GPIHBP1 is detectable in plasma or serum, and if so, to quantify the levels of GPIHBP1 in human blood. We developed 2 new rat mAbs against human GPIHBP1, IU-79, and IU-20 and used those antibodies to establish a sandwich immunoassay for human GPIHBP1. We used this assay to compare the GPIHBP1 blood levels in healthy subjects and in GPIHBP1-deficient subjects. We also examined GPIHBP1 levels in patients under treatment for cardiovascular or metabolic diseases.

## Methods

### Monoclonal antibody preparation

Complementary DNA of human GPIHBP1 was synthesized by FASMAC (Tokyo) and expressed in chinese hamster ovary cells. The recombinant GPIHBP1 from transfected chinese hamster ovary cells was purified by anti-flag M2 column. Wister rats were immunized with the purified recombinant human GPIHBP1. Antibody titers in the plasma of the immunized rats were monitored by enzyme-linked immunosorbent assay (ELISA), and hybridomas were generated by fusing splenocytes with X63 myeloma cells. After subjecting the hybridomas to selection with azaserine and hypoxanthine, samples of medium were screened for GPIHBP1 antibodies by ELISA. Seventeen hybridomas were expanded and subcloned. Five mAbs, including mAbs IU-79 and IU-20, were isotyped with commercial kits (BD Bioscience, Tokyo). The hybridomas were adapted to serum-free medium, and the mAbs were purified from the chinese hamster ovary cell culture medium on a protein G–agarose column. The antibodies were eluted from the protein G column with glycine•HCl (pH 2.5).

### Immunoblotting

Soluble versions of wild-type human GPIHBP1, GPIHBP1-W109S, and a mutant GPIHBP1 lacking the acidic domain, all containing an amino-terminal uPAR tag (detectable with mAb R24), were expressed in *Drosophila* S2 cells.[22] Recombinant human GPIHBP1 (2.0 μg) was size fractioned by sodium dodecyl sulfate–polyacrylamide gel electrophoresis under reducing and nonreducing conditions and transferred to a sheet of nitrocellulose. The blots were then incubated with rat mAbs IU-79 or IU-20 (5 μg/mL), followed by an IRDye-labeled donkey anti-rat IgG (Rockland, 1:2000). Western blots of nonreduced GPIHBP1 were also incubated with IRDye680-labeled mAb R24 (specific for uPAR, 1:500).[23] Blots of reduced samples were incubated with IRDye800-labeled mAb RF4 (which binds to the acidic domain of hGPIHBP1, 1:500).[16] Western blots were imaged with an infrared scanner (LI-COR).

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