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An ELISA for quantifying GPIHBP1 autoantibodies and making a diagnosis of the GPIHBP1 autoantibody syndrome

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ABSTRACT

Background: Autoantibodies against GPIHBP1, the endothelial cell transporter for lipoprotein lipase (LPL), cause severe hypertriglyceridemia ("GPIHBP1 autoantibody syndrome"). Affected patients have low serum GPIHBP1 and LPL levels. We report the development of a sensitive and specific ELISA, suitable for routine clinical use, to detect GPIHBP1 autoantibodies in serum and plasma.

Methods: Serum and plasma samples were added to wells of an ELISA plate that had been coated with recombinant human GPIHBP1. GPIHBP1 autoantibodies bound to GPIHBP1 were detected with an HRP-labeled antibody against human immunoglobulin. Sensitivity, specificity, and reproducibility of the ELISA was evaluated with plasma or serum samples from patients with the GPIHBP1 autoantibody syndrome.

Results: A solid-phase ELISA to detect and quantify GPIHBP1 autoantibodies in human plasma and serum was developed. Spiking recombinant human GPIHBP1 into the samples reduced the ability of the ELISA to detect GPIHBP1 autoantibodies. The ELISA is reproducible and sensitive; it can detect GPIHBP1 autoantibodies in samples diluted by > 1000-fold.

Conclusion: We have developed a sensitive and specific ELISA for detecting GPIHBP1 autoantibodies in human serum and plasma; this assay will make it possible to rapidly diagnose the GPIHBP1 autoantibody syndrome.

1. Introduction

GPIHBP1 (Glycosylphosphatidylinositol-anchored high density lipoprotein–binding protein 1) is a key player in intravascular lipolysis [1,2]. GPIHBP1 is expressed exclusively on capillary endothelial cells, and its main function is to bind lipoprotein lipase (LPL) within the subendothelial spaces and shuttle it across endothelial cells to its site of action in the capillary lumen [1,2]. *GPIHBP1* loss-of-function mutations abolish the ability of GPIHBP1 to bind and transport LPL, markedly reducing levels of LPL in capillaries and resulting in severe hypertriglyceridemia (chylomicronemia) and a substantial risk for acute pancreatitis [3–8]. Patients with GPIHBP1 mutations invariably have low levels of GPIHBP1 in serum and low levels of LPL in the pre- and post-heparin plasma (consistent with reduced delivery of LPL to the capillary lumen) [3–8].

Recent studies have unequivocally demonstrated that GPIHBP1 autoantibodies are responsible for some acquired cases of chylomicronemia [9,10]. Beigneux and coworkers identified six patients with unexplained chylomicronemia with GPIHBP1 autoantibodies ("GPIHBP1 autoantibodies syndrome") [9,10]. They demonstrated that the GPIHBP1 autoantibodies blocked the ability of GPIHBP1 to bind and transport LPL. Like patients with inherited forms of GPIHBP1 deficiency, patients with the GPIHBP1 autoantibody syndrome have low levels of LPL in their plasma [9,10]. The plasma levels of GPIHBP1 are also low because the GPIHBP1 autoantibodies interfere with the detection of GPIHBP1 in the plasma [9]. Many but not all patients with the GPIHBP1 autoantibody syndrome have clinical or serological evidence for autoimmune disease [9].

Abbreviations: LPL, lipoprotein lipase; GPIHBP1, glycosylphosphatidylinositol-anchored high density lipoprotein binding protein-1

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The prevalence of high TG patients (above 1000 mg/dl) is known about 1 /1000 cases in all over the world. Most of the autoantibodies positive cases reported [9,10] were found in high TG cases. However, the prevalence itself is very low, it is important to detect the autoantibodies among those high TG cases. Because the presence of autoantibodies often exhibits the autoimmune disease, the patients need to be treated differently from the general lipid disorders by such as fibrates but by immunosuppressive agents. At the current time, assays for GPIHBP1 autoantibodies are not routinely available.

2. Materials and methods

2.1. Blood samples

Blood samples were obtained according to the principles outlined in the Declaration of Helsinki. Sera from 28 healthy Japanese volunteers in the non-fasting state (aged 23–60 y, male 19, female 9) were obtained at Immuno-Biological Laboratory. We also examined de-identified archived plasma samples from patients with loss-of-function mutations in GPIHBP1 [8,9,11]. Those samples had been sent to UCLA without identifiers [9] and were deemed exempt from institutional review board approval. As positive controls, we used archived serum and plasma samples from documented cases of GPIHBP1 autoantibody syndrome about the details already reported [9,10]. Briefly, the patients with GPIHBP1 autoantibodies who had chylomicronemia had received a diagnosis of an autoimmune disease (SLE or Sjögren's syndrome). In such patients, autoantibodies against many proteins can develop, and our data indicate that GPIHBP1 is one of those proteins. Because of the transfer of maternal autoantibodies, an infant who was born to a mother with SLE was found to have neonatal lupus (characterized by cutaneous lesions and cardiac conduction abnormalities). On the basis of our data, chylomicronemia is a potential finding in infants born to mothers with SLE. Of the other patients with GPIHBP1 autoantibodies, 2 had no evidence of rheumatologic disease.

2.2. An ELISA system to detect GPIHBP1 autoantibodies

A cDNA encoding a soluble version of FLAG-tagged human GPIHBP1 (amino acids 21–150) was synthesized by FASMAC, expressed in CHO cells, and human GPIHBP1 protein was then purified from the medium with a FLAG M2 column. Plasma or serum samples (diluted 1000-fold) were added to wells of a 96-well ELISA plate that had been coated with the purified GPIHBP1 (50 ng/well, 1 h at room temperature) (Fig. 1). After washing, 50 ng of HRP-labeled goat anti-human IgG was added to the wells and incubated for 30 min at room temperature. After washing, 100 µl of TMB substrate was added to the wells. After 15 min, the reaction was stopped by adding 100 µl of 2 M sulfuric acid to the wells. The absorbance (OD) was read at 450 nm (Fig. 1).

Using a plasma sample from a well-documented case of the GPIHBP1 autoantibody syndrome [9,10], we set a GPIHBP1 autoantibody titer at 1 unit (U)/ml when a 1:1000 dilution of a sample by PBS yielded an OD of 2.0 (at 450 nm) (Fig. 2A). Further 1:2 serial dilutions of the sample by PBS revealed that the ELISA could detect GPIHBP1 autoantibodies when the titer fell within the range of 0.03 U/ml to 1 U/ml (Fig. 2A), and that the assay was linear up to a 1:4000 dilution of the plasma samples (Fig. 2B).

2.3. Measurements of triglycerides and GPIHBP1 in serum and plasma

Plasma triglyceride levels were determined with enzymatic assay (Quick Neo TG II, Shino-Test Corporation, Tokyo). Plasma GPIHBP1 levels were measured with a solid-phase monoclonal antibody–based sandwich ELISA (Immuno-Biological Labs. Fujioka, Japan) [12]. For the GPIHBP1 ELISA, serum and plasma were diluted 10-fold in PBS; 100 µl of the diluted samples were added to wells of the 96-well ELISA plate.

3. Results

3.1. Plasma sample processing

The serum or plasma samples were separated by centrifugation (2500 ×*g*) at 4 °C for 10 min and kept frozen at −80 °C until analysis. Heparin administration (30 units/kg) was performed to confirm the low LPL concentration in 3 GPIHBP1 autoantibodies positive cases.

3.2. Performance of the GPIHBP1 autoantibodies ELISA

Detection of GPIHBP1 autoantibodies titers was linear up to 1 U/ml (Fig. 2A) and the dilution to over 4000 fold (Fig. 2B). The analytical detection limit was estimated as the equal to the mean absorbance of 10 replicates of the zero calibrator plus 3 SD. The detection limit was as low as 0.03 U/ml.

The clinical relevance of the QC levels were tested using the following QC samples for determining the clinical decision point. The low QC sample of GPIHBP1 autoantibodies was diluted 4000 fold (OD 0.75mAb) of the GPIHBP1 autoantibodies positive plasma (1 U/ml). Middle QC sample 2000 fold dilution (OD 1.2 mAb) and high QC sample 1000 fold dilution (OD 2.0 mAb). Negative cases were below low QC samples at 1000 fold dilution which was below 0.1 U/ml (OD 0.20 mAb). All positive cases were above OD 0.5 mAb at 450 nm.

The intra- and inter-assay variation of the GPIHBP1 autoantibodies ELISA was measured with three quality control (QC) samples, corresponding to the high, middle, and low region of the calibration curve. The intra-assay variation was calculated from four repeated measurements of the three QC samples in a single plate. The inter-assay variation was calculated by measuring each QC sample across four different

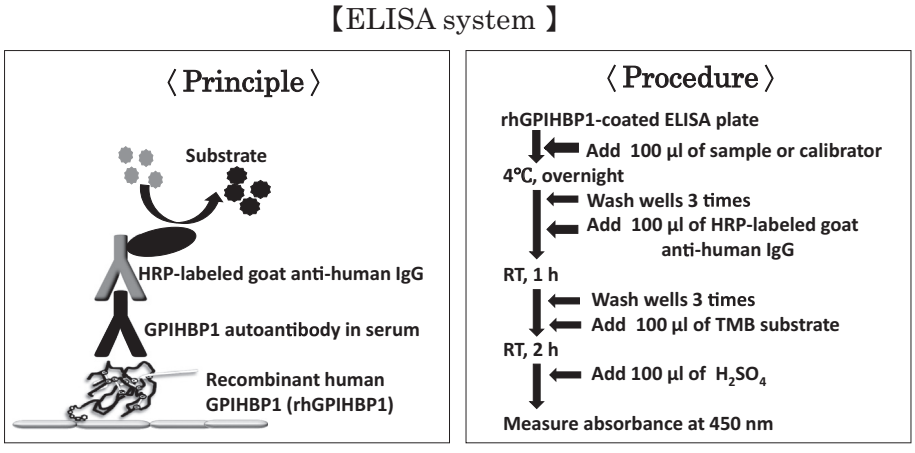

Fig. 1. An immunoassay for quantifying autoantibodies against GPIHBP1. Human plasma or serum is added to wells of an ELISA plate that had been coated with recombinant human GPIHBP1. After washing, human immunoglobulins (IgGs) bound to the recombinant human GPIHBP1 are detected with an HRP-labeled goat anti-human IgG antibody.