and HDL-C concentrations, but none with CVD *(21–23)*. Thus, genetic variation in EL can modulate plasma HDL-C concentrations, although the relationship with CVD remains controversial. Although EL is still expected to be an attractive pharmacological target for raising HDL-C concentrations, the clinical significance of plasma EL concentration on plasma HDL-C concentrations and atherosclerosis in humans has not been fully elucidated due to the lack of a standard and reliable assay system. To obtain a better understanding of the association of EL and plasma concentrations of HDL-C in humans, our goal was to establish a new assay system for EL mass measurement using newly generated antibodies.

Materials and Methods

MONOCLONAL ANTIBODY PREPARATION

We transfected Chinese hamster ovary (CHO) cells (American Type Culture Collection) with the human EL-c-myc/pHBAP-3-neo plasmid *(10)* and selected stable transfectants with 500 μg/mL G418 (Invitrogen). We screened EL expression in the stable transfectants using an ELISA system with monoclonal antibodies against EL (clones 11–9B and 2–12E) *(16)* and chose 1 of the high expression clones, referred to as hEL-myc/CHO 53A5, for subsequent experiments. The cells were cultured for 24 h in TIL medium (Immuno-Biological Laboratories) containing 10% fetal bovine serum (FBS) (PAA Laboratories), after which the medium was changed for serum-free TIL medium. After 3 days, the supernatants were collected and measured. We purified EL protein from the concentrated conditioned medium of hEL-myc/CHO 53A5 using an immunoaffinity column containing anti-EL carboxy-terminus antibody (clone 2–12E) *(14, 16)*, emulsified with Freund complete adjuvant, and then immunized into BALB/c mice (Charles River). After a boost with immunogen, we carried out fusion of spleen cells with X63-Ag8.653 myeloma cells (Immuno-Biological Laboratories) with PEG1500 (Roche Applied Science) followed by screening for desired hybridoma reactive only to EL by immunoblotting. We identified 2 monoclonal antibodies that reacted with amino terminus (26A1) and carboxy terminus (48A1) of EL among 15 clones.

PREPARATION OF EL CALIBRATORS AND OTHER LIPASES

To obtain standard full-length EL protein, the concentrated culture medium of hEL-myc/CHO 53A5 was incubated with 70% NH_4SO_4, and then put through immuno-affinity columns containing monoclonal antibodies against amino (clone 5–3B) and carboxy (clone 2–12E) terminus of EL *(14, 16)*. We estimated the purity of the recombinant hEL protein by densitometry using a Multi Gauge (Fujifilm) and determined the concentration of the protein by comparison with BSA (PAA Laboratories) as an indicator after electrophoresis. In addition, we used the culture supernatant of hEL-myc/CHO 53A5 cells as a working standard for the ELISA system.

To test the cross-reactivity of the ELISA with other lipase members, including lipoprotein lipase (LPL) and hepatic triglyceride lipase (HTGL), we purchased recombinant human LPL protein from BioVendor and generated recombinant human HTGL as follows. Full-length human HTGL cDNA was identified in human liver cDNA (Clontech) by PCR using primers 5′-ATCGGAGAAATGGACAC AAGTCCC-3′ and 5′-CGCTCGAGTCTGATCTTT CGCTTTGATGTTTT-3′. A FLAG-epitope tag was added to the 3′ end of cDNA before subcloning. To generate the plasmid encoding hHTGL-Full, the cDNA was inserted into pcDNA3.1(+) expression vector (Invitrogen). Human HTGL exhibits cell surface binding through the 5 carboxyl-terminal residues (KRKIR) *(24)*. Therefore, to promote secretion to a culture supernatant, we prepared a truncated human HTGL mutant (hHTGL-471) by deleting the 5 carboxyl-terminal residues. We used antisense PCR primer (5′-CGCTCGAGTGATGTTTTAGACTTTATTTCACA-3′) to generate the plasmid encoding hHTGL-471. The PCR product was inserted into pcDNA3.1(+) expression vector after a FLAG-epitope tag was added to the 3′ end.

CHO cells were transfected with the hHTGL-471 plasmid and selected with 500 μg/mL G418 to establish stable transfectants. We screened hHTGL-471 expression in the transfectants by using anti–c-FLAG rabbit IgG (Immuno-Biological Laboratories) and generated 1 of the high-expression clones, referred to as human HTGL-471/CHO 3B1. We purified HTGL protein from the concentrated conditioned medium of human HTGL-471/CHO 3B1 using an anti-FLAG M2 affinity gel (Sigma-Aldrich). We estimated the purity of the recombinant human HTGL protein by densitometry using a Multi Gauge and determined the concentration of the protein by comparison with BSA as an indicator after electrophoresis.

IMMUNOBLOTTING AND IMMUNOPRECIPITATION-IMMUNOBLOTTING

We analyzed the supernatant from hEL-myc/CHO 53A5 cells by SDS-PAGE, followed by staining with Coomassie Brilliant Blue. For immunoblotting, 5 μL conditioned medium of hEL-myc/CHO 53A5 was used for 12% SDS-PAGE and transferred to nitrocellulose membrane (Bio-Rad). The membrane was incubated with 2 μg of the 26A1 or 48A1 antibody followed by incubation with secondary antibody conjugated with

horseradish peroxidase (HRP) (1:4000, Immuno-Biological Laboratories). We assessed the reactivity of antibodies by immunoprecipitation-immunoblotting. The supernatant from the hEL-myc/CHO 53A5 cells was incubated with 2 μg 26A1 or 48A1 or mouse IgG (as negative control) and then with added Protein-G Sepharose (GE Healthcare Japan). After further incubation, the supernatant was centrifuged, and the resulting pellet was washed 3 times with 500 μL TNE buffer (10 mmol/L Tris-HCl, pH 7.8, 150 mmol/L NaCl, 1 mmol/L EDTA, 1% Nonidet P-40). The pellet was subsequently lysed, and we performed immunoblot analysis using biotinylated anti-EL amino-terminus monoclonal antibody (clone 11–9B) *(14, 16)* followed by HRP-conjugated streptavidin system. We visualized the EL signal by use of an ECL reagent (Amersham Biosciences).

IMMUNOFLUORESCENCE

The hEL-myc/CHO 53A5 and control mock-CHO cells cultured on coverslips were washed with prewarmed PBS (137 mmol/L NaCl, 10 mmol/L phosphate, 2.7 mmol/L KCl, pH 7.4), fixed with 4% paraformaldehyde in PBS for 15 min, and permeabilized with 0.1% Triton X-100 for 60 min. After saturation of unspecific sites with 100 g/L BSA/PBS, the cells were first incubated with the EL 26A1 or 48A1 antibody (1:200) or negative control (mouse mAb IgG Isotype Control, Cell Signaling Technology), and then with fluorescence-labeled secondary antibody (Alexa Fluor594 goat anti-mouse IgG, Invitrogen, 1:200). DAPI was used for nuclear staining. Images were captured by use of the Biozero BZ-8000 microscope (Keyence).

EL SANDWICH ELISA PROTOCOL

Microtiter plates (96 wells) were coated by adding 100 μL of 100 mmol/L carbonate buffer (pH 9.5) to each well that also contained 1.0 μg purified 48A1 mouse monoclonal IgG, followed by incubation overnight at 4 °C. The plates were then washed with PBS-T and blocked with 200 μL of 1% (wt/vol) BSA in PBS containing 0.05% NaN_3/well overnight at 4 °C. After two washings with PBS-T, test samples and recombinant EL, as a standard, that had been serially diluted with 1% BSA in PBS-T per 100 μL were added to the wells of the coated microtiter plate in duplicate and incubated at 4 °C overnight. After 4 washes with PBS-T, 100 μL HRP-conjugated 26A1 mouse IgG Fab′ was added to each well and the samples were incubated for 30 min at 4 °C. The wells were washed 5 times with PBS-T, and 100 μL tetramethyl benzidine solution (Kem-En-Tec) was added to each well as a substrate, followed by incubation in the dark for 30 min at room temperature. The reaction was terminated by adding 100 μL of 0.5 mol/L H_2SO_4. We measured absorbance of the solution at 450 nm by means of an ELISA reader (E-Max; Molecular Devices).

To assess the intra- and interassay precision for the ELISA, we established 3 QC samples covering the high, middle, and low range of the calibration curves. We determined intraassay precision by 24 repeated measurements of each QC sample in a plate, and interassay precision by assessing each QC sample across 6 different plates with quintuple wells. Additionally, for assessing the recovery rate in blood samples, different concentrations of recombinant EL added to samples were measured, and the recovery rate was validated as the difference between the measured concentration and the theoretical concentration. The analytical limit of quantification for this kit was determined on the basis of the guidelines provided by CLSI evaluation protocols.

The ELISA assay system was finally designed as a kit (Immuno-Biological Laboratories, code 27182).

PREPARATION OF BLOOD SAMPLES

The investigation conformed to the principles outlined in the Declaration of Helsinki, and the clinical study was approved by the Institutional Review Board of Kobe University Graduate School of Medicine.

We conducted the first set of experiments to determine the effect of heparin administration on plasma EL mass. We collected pre- and postheparin plasma samples from overweight and obese participants in a nutritional research study conducted at the University of California, Davis Clinical and Translational Science Center's Clinical Research Center as described previously *(25)*. The activity of HTGL in the plasma samples was measured as described previously *(26)*.

The second set of experiments was conducted to evaluate EL mass in patients with diagnosed existing CVD. Whole blood was obtained from 645 Japanese patients consecutively admitted to Kobe University Hospital, Kobe, Japan, from April 2008 to March 2011, with written informed consent. Because it has been reported that preheparin EL mass is significantly correlated with postheparin EL mass, blood was collected without administration of heparin in the fasting state, while both pre- and postheparin plasma was obtained from some patients. The sera and plasma were immediately separated and kept frozen at −80 °C until assay. In some preliminary experiments, we obtained plasma and sera of healthy subjects (Veritas). Plasma concentrations of HDL-C, LDL-C, and TG were measured enzymatically.

STATISTICAL ANALYSIS

We conducted statistical analysis with Stat View version 5.0 (SAS Institute). We used Spearman correlation coefficient analysis to assess associations between measured parameters. Results are expressed as mean (SE), and $P < 0.05$ was considered significant.

Results

IDENTIFICATION OF RECOMBINANT hEL AND CHARACTERIZATION OF ANTIBODIES AGAINST EL

We generated a pair of antibodies recognizing the amino terminus (26A1) and carboxy terminus (48A1) that exhibited a highly specific reactivity with the EL protein derived from hEL-myc/CHO 53A5 cells. Immunoblotting revealed a strong signal for 68-kDa mature EL protein (Fig. 1A). To confirm whether the antibodies had the ability to react against native EL in aqueous conditions, we performed an immunoprecipitation-immunoblotting analysis. Both 26A1 and 48A1 antibodies were able to immunoprecipitate EL originated from the conditioned medium of hEL-myc/CHO 53A5 (Fig. 1B). Immunofluorescence revealed that EL expression was abundantly detected in the cytosol of hEL-myc/CHO 53A5 (Fig. 1C). No signal was detected in negative controls, i.e., mock-transfected cells with primary antibodies or hEL-myc/CHO 53A5 cells without primary antibodies. Thus, we were able to identify EL expression in culture medium of hEL-myc/CHO 53A5 cells and confirm the specific reactivity of the 26A1 and 48A1 antibodies with EL protein.

SPECIFICITY, RECOVERY, AND IMPRECISION OF THE ELISA

Because the 26A1 and 48A1 antibodies had a highly specific reactivity with the EL protein, we chose them for establishment of the new sandwich ELISA system. The standard dose–response curve for the EL ELISA system exhibited a linear shape when plotted on a log/log scale over a range from 31 to 2000 pg/mL, and the linearity was excellent ($R^2 = 0.99$) (Fig. 2A). The new ELISA system worked for both serum and EDTA plasma samples equally. Although EL has a 44% and 41% amino acid sequence homology with LPL and HTGL *(10)*, the cross-reactivity of this ELISA against human LPL and HTGL was <0.1% (Fig. 2B). Imprecision was determined with 3 supplemented QC controls (high, middle, and low). The intraassay imprecision exhibited CVs of 1.9% in the high, 2.7% in the middle, and 3.0% in the low controls (Table 1). Additionally, the interassay results for the CVs were 3.7% in the high, 2.0% in the middle, and 2.8% in the low controls. Thus, we considered the ELISA system to be reliable from the standpoint of imprecision. The recoveries were >85.6% for human EDTA plasma samples at 4× dilution, >74.7% for human serum, and nearly 100% for TIL media supplemented with 10% FBS (Table 2). We calculated the assay limit of quantification as 5.7 pg/mL using CLSI protocols.

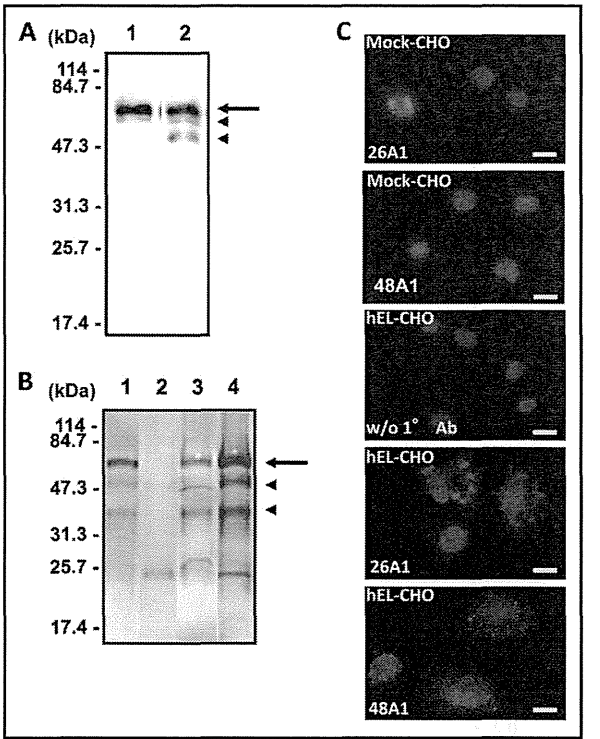

Fig. 1. Immunoblotting of EL protein with anti-EL monoclonal antibodies.

(A), A strong signal of full-length EL (68 kDa) was detected with both 48A1 (*lane 1*) and 26A1 (*lane 2*) anti-EL antibodies, whereas smaller-sized minor bands were also detected with 26A1. (B), Twenty-fold concentrated culture medium of hEL-myc/CHO 53A5 (*lane 1*), EL protein immunoprecipitated from culture supernatant of hEL-myc/CHO 53A5 with mouse IgG (negative control, *lane 2*), with 48A1 (*lane 3*), or with 26A1 (*lane 4*) were detected. (C), Expression of EL (red) in hEL-myc/CHO 53A5 (hEL-CHO) cells was evaluated by immunofluorescence with the 48A1 or 26A1 antibodies. DAPI (blue) is for nuclear stain. Mock-transfected (Mock-CHO) cells treated with the antibodies and hEL-myc/CHO 53A5 in which the primary antibody was replaced by nonspecific IgG (w/o 1° Ab) are shown as negative controls. Scale bar indicates 10 μm.

Because EL has several heparin-binding domains, we investigated the effect of heparin administration on plasma EL mass. Unexpectedly, there was no significant difference in EL mass between pre- and postheparin samples (Fig. 3, A and B), in contrast to the marked heparin-releasable HTGL activity in the same samples

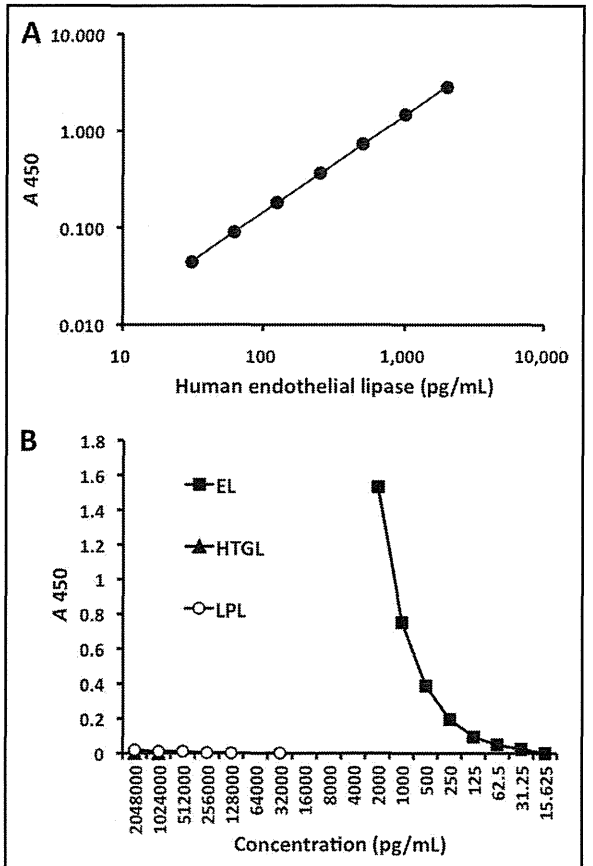

Fig. 2. Characteristic of the hEL ELISA system.
(A), Calibration curve for calculation of EL concentrations. (B), Specificity of the EL ELISA system. A, absorbance.

(Fig. 3A). Although there are heparin-binding sites in the carboxy-terminal end of EL, we confirmed that heparin does not block the binding sites of the amino-terminus (26A1) or carboxy-terminus (48A1) antibody.

Table 1. Intra- and interassay imprecision.

QC	Measured value, pg/mL	SD, pg/mL	CV, %	n
Intraassay imprecision				
High	1126.9	20.85	1.9	24
Middle	285.9	7.71	2.7	24
Low	90.8	2.69	3.0	24
Interassay imprecision				
High	1100.7	40.81	3.7	6
Middle	282.7	5.58	2.0	6
Low	91.4	2.54	2.8	6

Table 2. Recovery validation.

Sample	Theoretical value, pg/mL	Measured value, pg/mL	% Recovery
Human plasma	1147.9	982.7	85.6
	647.9	557.9	86.1
	397.9	357.4	89.8
Human serum	1098.6	820.6	74.7
	598.6	449.3	75.1
	348.6	274.3	78.7
TIL media supplemented with 10% FBS	1000.0	941.1	94.1
	500.0	483.0	96.6
	250.0	245.7	98.3

INVERSE CORRELATION OF SERUM EL MASS AND HDL-C LEVELS IN CVD

The serum EL mass in 645 consecutive human subjects was 344.4 (7.7) pg/mL, and ranged from 55.2 to 1387.7 pg/mL. No patients had an EL concentration below the limit of quantification. The distribution of EL mass was skewed to the left (Fig. 4A). The EL mass was not correlated with serum HDL-C (Fig. 4B and Supplemental Fig. 1B, which accompanies the online version of this article at http://www.clinchem.org/content/vol58/issue12) or LDL-C concentrations (data not shown) in this population. Because our previous study showed that EL mass was associated with plasma HDL-C concentrations in patients with CVD *(16)*, we next investigated the serum EL mass in patients having atherosclerotic CVD. The EL mass concentration in these 228 patients with CVD was 395.8 (15.1) (range 57.7–1387.7) pg/mL, which was significantly higher than that in the 645 consecutive patients ($P < 0.001$), and the EL distribution was again skewed to the left (Fig. 5A). Concomitantly, the patients with CVD had significantly lower concentrations of serum HDL-C than those without CVD {46.2.0 (1.0) vs 52.0 (0.6) mg/dL [1.20 (0.03) vs 1.35 (0.02) mmol/L]}, $P < 0.001$). When serum EL concentration was compared to the lipid profile in the CVD patients, it was inversely correlated with plasma HDL-C concentrations ($R = -0.250$, $P < 0.001$) (Fig. 5B and online Supplemental Fig. 2B), but not with LDL-C ($R = -0.055$, NS), or triglyceride ($R = 0.078$, NS) concentrations.

Discussion

We generated specific EL monoclonal antibodies against recombinant EL that reacted with the amino (26A1) and carboxy (48A1) terminus. Both antibodies had strong reactivity with native EL protein and en-

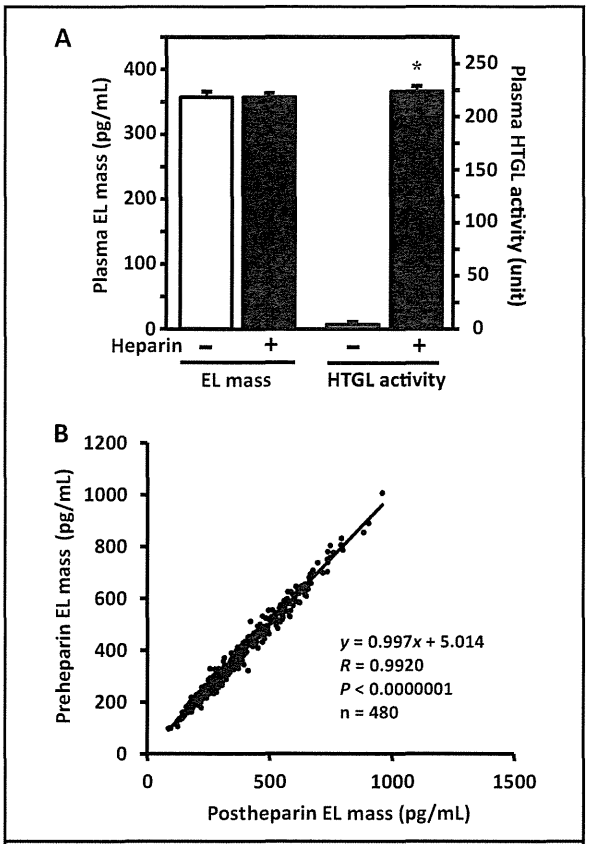

Fig. 3. Effect of heparin administration on plasma EL mass.

(A), Administration of heparin did not affect plasma EL mass but markedly increased plasma HTGL activity in the same pre- and postheparin samples (n = 480). *$P < 0.001$ vs. without heparin. (B), Scatter plot showing association between pre- and postheparin EL mass.

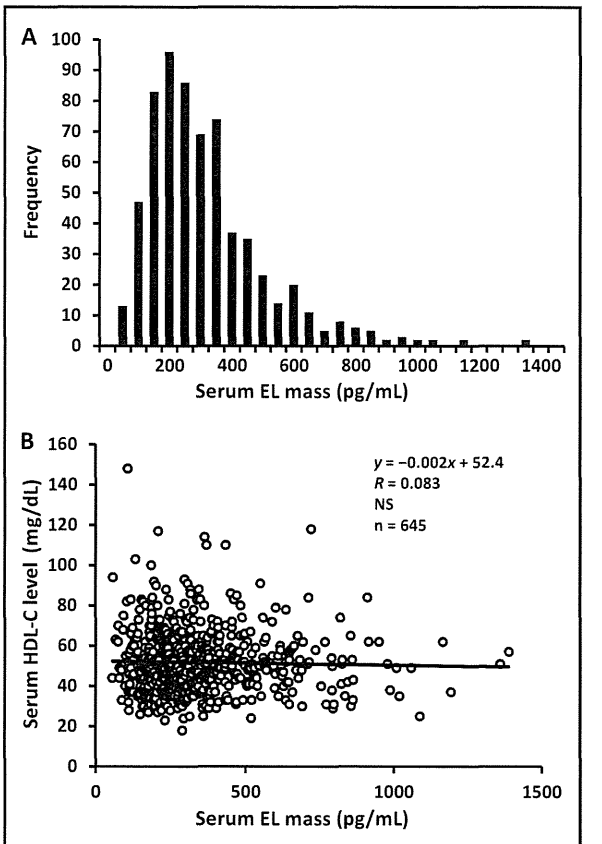

Fig. 4. Serum EL mass and HDL-C concentrations in all patients.

(A), Histogram showing the skewed distribution of EL mass (n = 645). (B), Lack of correlation of serum EL mass with serum HDL-C concentrations. NS, not statistically significant.

abled the detection of full-length EL by the sandwich ELISA. The limit of quantification of 5.7 pg/mL for hEL is much lower than that of previous ELISA systems, probably because the new antibodies are more specific for full-length EL protein than the old ones, which were generated against peptide fractions of EL *(16)*. The serum EL mass in preheparin plasma was approximately 70–1000 pg/mL in the present study. Previous studies by our group and another group reported that the concentrations of EL mass in preheparin plasma were approximately 10–1000 μg/L, concentrations approximately 1000-fold higher than measured with the new assay *(15, 16)*. These differences have resulted in confusion as to whether the plasma concentration of EL is really higher than the concentrations of LPL and HTGL without heparin infusion. The concentrations of other lipases, including LPL and HTGL, in preheparin plasma have been reported to be <30~100 μg/L *(27, 28)*. When compared with the low plasma concentrations of other lipase members, we speculate that the range of EL concentrations determined by the new ELISA is more reasonable than those by the previous one. We consider the specificity of the antibodies used for this assay to be suitable for determining specific EL mass concentrations in human plasma, which showed different reactivity from the antibodies generated against peptide fractions in EL *(16)*. The new ELISA may also be useful for identifying cases of genetic deficiency of EL in humans.

In the present study, a modest but significant inverse correlation between serum EL and HDL-C concentrations in patients with CVD was noted, whereas the relationship was not observed in all patients. The EL concentration was not correlated with serum LDL-C or TG concentrations. These findings confirmed the previous notion that EL is a determinant of

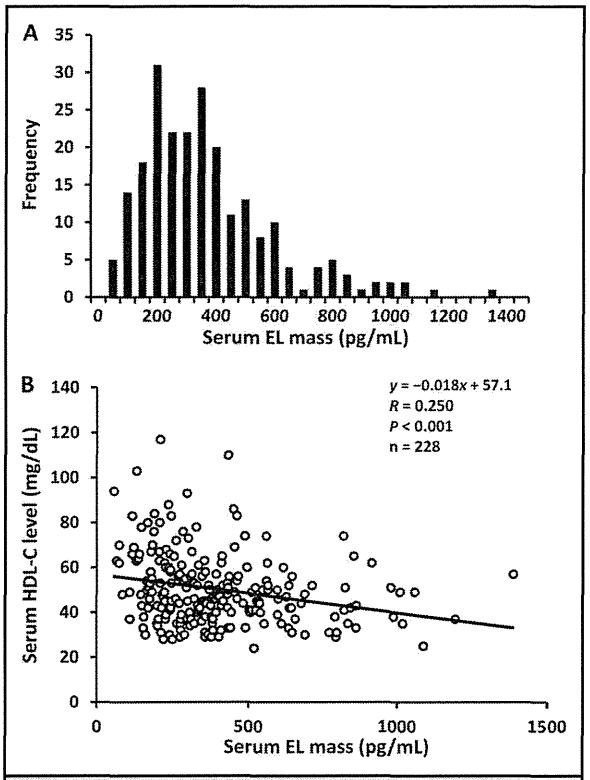

Fig. 5. Serum EL mass and HDL-C concentration in patients with cardiovascular disease.

(A), Histogram showing the skewed distribution of EL mass (n = 228). (B), Inverse correlation of serum EL mass with serum HDL-C concentrations.

plasma HDL-C concentrations, particularly in patients with CVD. It has been reported that EL expression is highly regulated by a variety of factors including inflammatory cytokines, biomechanical forces, lipopolysaccharide, angiotensin II, and oxidized LDL *(29–31)*. In fact, plasma EL mass was increased in experimental endotoxemia in humans and correlated with inflammatory markers such as C-reactive protein, interleukin-6, and secretory phospholipase A2-IIa *(32, 33)*. In contrast, statins reduce EL expression and plasma EL mass *(16, 34)*, which is accompanied by increased plasma HDL-C concentrations in humans *(16)*. These findings suggest that a change in EL expression associated with inflammatory states may at least in part account for the variation of HDL-C concentrations in CVD patients.

Cell culture experiments revealed that cytokine-stimulated EL expression was concomitant with an increase in EL activities *(35)*. However, EL activity is partly regulated through posttranscriptional mechanisms. It has been reported that EL forms a head-to-tail dimer in the human plasma, and the homodimer formation is essential for the maintenance of EL activity *(36)*, as is the case with LPL and HTGL. In addition, EL is proteolytically processed into 40- and 28-kDa fragments and inactivated by proprotein convertases *(37)*. In this regard, our sandwich ELISA system can recognize the dimer of full-length EL with the intact enzymatic activity. On the other hand, angiopoietin-like 3 is known to act as an endogenous EL inhibitor *(38)*. In addition, human heat-inactivated serum inhibited EL phospholipase activity *(39)*, indicating the existence of some endogenous EL inhibitor in human serum. Furthermore, a naturally occurring variant in the EL gene (*LIPG*), glycine-26 to serine, which is associated with increased HDL, exhibits impaired synthesis *(20)*.

It has been reported that EL has several heparin-binding domains and binds to heparan sulfate proteoglycans on the vascular endothelium *(12, 15, 40)*. Therefore, EL should be released into plasma by heparin treatment *(15)*. In the present study, however, there was no difference in EL mass between pre- and postheparin plasma samples, whereas control HTGL activity was markedly increased by the heparin administration. When we evaluated EL mass by our previous ELISA system *(16)*, we confirmed that the administration of heparin did not affect plasma the EL protein. Thus, the interaction of EL with heparan sulfate proteoglycans needs to be determined by further studies.

In conclusion, we developed a sandwich ELISA using newly generated monoclonal antibodies specific to human plasma EL. The limit of quantification, range of linearity, and imprecision for EL quantification are suitable for both experimental and clinical use. From the preliminary study of a healthy reference range, we found that reference-range EL concentrations were between approximately 50 and 1400 pg/mL in human plasma. This range is much lower and, we suggest, more reasonable than the range measured with previous EL assays. It is possible that patients with low (EL deficiency) and high serum EL concentrations associated with high or low HDL-C concentrations will be identified by use of this new assay for EL.

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Special Report

Diagnosis and Management of Type I and Type V Hyperlipoproteinemia

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Both type I and type V hyperlipoproteinemia are characterized by severe hypertriglyceridemia due to an increase in chylomicrons. Type I hyperlipoproteinemia is caused by a decisive abnormality of the lipoprotein lipase (LPL)- apolipoprotein C-II system, whereas the cause of type V hyperlipoproteinemia is more complicated and more closely related to acquired environmental factors. Since the relationship of hypertriglyceridemia with atherosclerosis is not as clear as that of hypercholesterolemia, and since type I and V hyperlipoproteinemia are relatively rare, few guidelines for their diagnosis and treatment have been established; however, type I and V hyperlipoproteinemia are clinically important as underlying disorders of acute pancreatitis, and appropriate management is necessary to prevent or treat such complications. Against such a background, here we propose guidelines primarily concerning the diagnosis and management of type I and V hyperlipoproteinemia in Japanese.

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Key words; Chylomicronemia, Gene mutation, Hyperlipidemia, Lipase, Triglyceridemia

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Background

According to Fredrickson's classification of hyperlipoproteinemia (WHO classification), type I and V hyperlipoproteinemia (hyperlipidemia) are characterized by an increase in chylomicrons alone and an

increase in very low-density lipoprotein (VLDL) in addition to chylomicrons, respectively[1]. Type I hyperlipoproteinemia is a clinical condition showing the severest hypertriglyceridemia and is classically represented by two rare genetic disorders, i.e., familial lipoprotein lipase (LPL) deficiency (MIM 238600) and familial apolipoprotein C-II deficiency (MIM 207750)[2]. Even rarer conditions such as familial inhibitor of lipoprotein lipase (MIM 118830) and the presence of autoantibodies also cause type I hyperlipoproteinemia[3, 4]. More recently, patients with mutations in two additional genes have also been reported to manifest primary type I hyperlipoproteinemia, i.e., genes for glycosylphosphatidylinositol-anchored high density lipoprotein-binding protein 1 (GPIHBP1) (MIM 612757) and for lipase maturation factor 1 (LMF1) (MIM 611761)[5, 6]. Since LPL is an insulin-dependent enzyme, diabetic lipemia observed in insulin-deficient conditions such as type 1 diabetes is well-known as secondary type I hyperlipoproteinemia. Therefore, type I hyperlipoproteinemia is caused by a decisive abnormality of either LPL, which is a rate-limiting enzyme involved in the hydrolysis of triglyceride (TG)-rich lipoproteins such as chylomicrons and VLDL, or apolipoprotein C-II, a cofactor necessary for the expression of LPL activity.

The cause of type V hyperlipoproteinemia is more complicated, and more miscellaneous clinical conditions are considered to belong to this category. It rarely shows familial occurrence, but its inheritance pattern is variable; therefore, type V hyperlipoproteinemia is usually considered to be triggered by acquired environmental factors in individuals with some congenital susceptibility to altered TG metabolism (genetic factors). While the involved environmental factors vary, involvement of heavy drinking, type 2 diabetes, hormonal therapy using steroids and estrogen, and drugs such as diuretics and β-blockers are frequently observed[7].

Many guidelines concerning the diagnosis and treatment of hypercholesterolemia have been formulated[8], and outstanding results of clinical intervention using lipid-lowering drugs, particularly statins, have been reported by large-scale clinical studies. On the other hand, since the relationship of hypertriglyceridemia with atherosclerosis is not as clear as that of hypercholesterolemia, and since type I and type V hyperlipoproteinemia, in particular, are relatively rare, few guidelines for their diagnosis and treatment have been established either in Japan or abroad; however, diagnostic criteria for primary hyperchylomicronemia were issued in the 1988 report by the Study Group on Primary Hyperlipidemia of the Ministry of Health and Welfare (Group leader: Seiichiro Tarui)[9]. Type I and V hyperlipoproteinemia are important as underlying disorders of acute pancreatitis, which is often lethal, and appropriate management, including restriction of fat intake, is necessary to prevent or treat such complications. Against such a background, the Study Group on Primary Hyperlipidemia of the Ministry of Health, Labour and Welfare (Group leader: Nobuhiro Yamada) proposes guidelines primarily concerning the diagnosis and management of type I and V hyperlipoproteinemia in Japanese.

Characteristics of Hyperchylomicronemia

The half-life of chylomicrons is about 5 minutes, and no chylomicron is observed in the plasma of normotriglyceridemic to moderately hypertriglyceridemic individuals after 12-hour fasting. Chylomicrons are considered to appear in fasting plasma in those with a serum TG level of about 1,000-2,000 mg/dl or above, and physical symptoms usually occur above this level ($\geq$ 2,000 mg/dl); therefore, there is a strict viewpoint defining hyperchylomicronemia as a serum TG level of 2,000 mg/dl or above accompanied by characteristic complaints or findings. However, caution is necessary, because there are patients showing no clinical symptom even at a serum TG level of 20,000-30,000 mg/dl, even though they are rare. From a clinical standpoint, it must be explained to the patient that there is risk of pancreatitis when the TG level is 1,000 mg/dl or higher even on casual sampling. This may also apply to neonates whose blood sampling after a long period of fasting is usually difficult. It must also be remembered in clinical laboratory testing that a marked increase in the serum TG level often affects the measurement system, causing apparently low serum amylase, hemoglobin, and electrolyte levels (e.g., sodium appears to be reduced by about 2-4 mEq/l with every 1,000 mg/dl increase in the TG). In particular, acute pancreatitis secondary to hypertriglyceridemia must not be misdiagnosed due to apparently low serum amylase.

Type I Hyperlipoproteinemia

A) Familial Lipoprotein Lipase (LPL) Deficiency

a) Concept and Definition

LPL is an enzyme that hydrolyzes TG of lipoprotein particles in blood, and its abnormal activity underlies type I hyperlipoproteinemia in many cases and type V hyperlipoproteinemia in some. Familial LPL deficiency is a rare monogenic disorder that exhibits the severest hyperchylomicronemia. It was first docu-

mented in 1932 in a boy born to a family with a history of consanguineous marriage[10], and the underlying abnormality was demonstrated to be a congenital defect of LPL activity, the rate-limiting enzyme of chylomicron hydrolysis, by Havel *et al.* in 1960[11]. Following the classification of familial hypercholesterolemia, it has been proposed to classify this disease as a class I defect causing complete loss of LPL protein, a class II defect characterized by the production of catalytically inactive protein, and a class III defect characterized by the production of inactive protein lacking affinity to heparan sulfate[12].

b) Etiology

The disease is caused by an abnormality of the human LPL gene, and the patients are homozygotes (including so-called compound heterozygotes) who have inherited LPL gene abnormalities from both parents in an autosomal recessive pattern with penetrance of 100%. The human LPL gene is located on the short arm of chromosome 8 (8p22), is about 35 kb in length, contains 10 exons, and codes for an enzyme protein consisting of 448 amino acids[13-15].

c) Clinical Symptoms

This disease is a relatively rare autosomal recessive disorder, and more than 30 families with this condition have been reported in Japan. The frequency of the occurrence of homozygous patients is estimated to be 1 in every 500,000 to 1 million people. Many patients have a family history of consanguineous marriage, and since patients exhibit chylous serum due to hyperchylomicronemia from early childhood and abdominal pain due to pancreatitis after the intake of fat, the disease is frequently diagnosed during the suckling period or early childhood. In females, the detection of hyperchylomicronemia during pregnancy may lead to the diagnosis. Attacks of abdominal pain due to acute pancreatitis following hyperchylomicronemia are often mistaken for acute abdomen, and the patient may undergo unnecessary laparotomy. While some patients acquire a dietary habit to avoid the intake of fat and suffer growth impairment, some show no marked attack of abdominal pain until adulthood, with consequent overlooking of the disease. It is the primary disease to be differentially diagnosed in a patient with persistent abdominal pain accompanied by hypertriglyceridemia[2].

Hyperchylomicronemia itself is also a major clinical finding, and the serum TG level reaches about 1,500 to even 20,000 mg/dl or more. The presence of chylomicrons can be confirmed by a simple method, i.e., the appearance of a top white cream layer in serum after standing at 4℃ for 24 hours or mild centrifugation. In typical cases, the lower layer is clear and transparent, reflecting an increase in chylomicrons alone. The possibility of LPL deficiency is high if the serum TG level is 1,500 mg/dl or higher, and the serum total cholesterol level is about 1/10 the serum TG level or lower. All other clinical findings are due to the marked increase in chylomicrons. First, eruptive xanthomas, which appear when the serum TG level increases to 2,000 mg/dl or above, are noted in about half of the patients, particularly on the extensor sides of the limbs, buttocks, and shoulders. They appear in association with changes in the serum TG level and disappear gradually over several weeks to a few months. When the serum TG level increases above 4,000 mg/dl, lipemia retinalis, in which the retinal vessels appear whitish pink due to chylous serum on funduscopy, appears, but vision is not impaired. Among other findings, hepatosplenomegaly due to the infiltration of macrophage foam cells that have phagocytozed lipids in the extravascular space, is observed, with hepatomegaly being frequent, but these changes are reversible and are rapidly improved (within 1 week) with correction of the serum lipid levels; however, the most serious complication is acute pancreatitis, and it must be managed carefully as it may be a prognostic determinant. From a clinical viewpoint, the possibility of acute pancreatitis must be explained to the patient if the TG level is 1,000 mg/dl or higher even on casual sampling. Dyspnea and neurological symptoms such as dementia, depression, and memory disorders have been reported as complications of this disorder.

As mentioned above, a major prognostic determinant of homozygous familial LPL deficiency is acute pancreatitis, which is often lethal. LPL deficiency has long been considered not to be closely related to atherosclerosis in humans, because no marked atherosclerotic lesion was noted at the autopsy of several homozygous patients with LPL deficiency who died due to acute pancreatitis. However, detailed research has reported that heterozygotes, which are considered to occur in 1 in every 500 individuals, usually show no marked abnormality in the lipid level but are likely to exhibit hypertriglyceridemia when they develop diabetes or are exposed to burdens such as severe obesity, excessive drinking, and pregnancy[16, 17]. There have also been reports of the frequent occurrence in heterozygotes of familial combined hyperlipidemia (FCHL)[12] and monogenic familial hypertriglyceridemia[16], which are common hyperlipidemia related to atherosclerosis; however, it remains controversial whether homozygotes with LPL gene abnormality are likely to develop atherosclerosis. A Canadian group

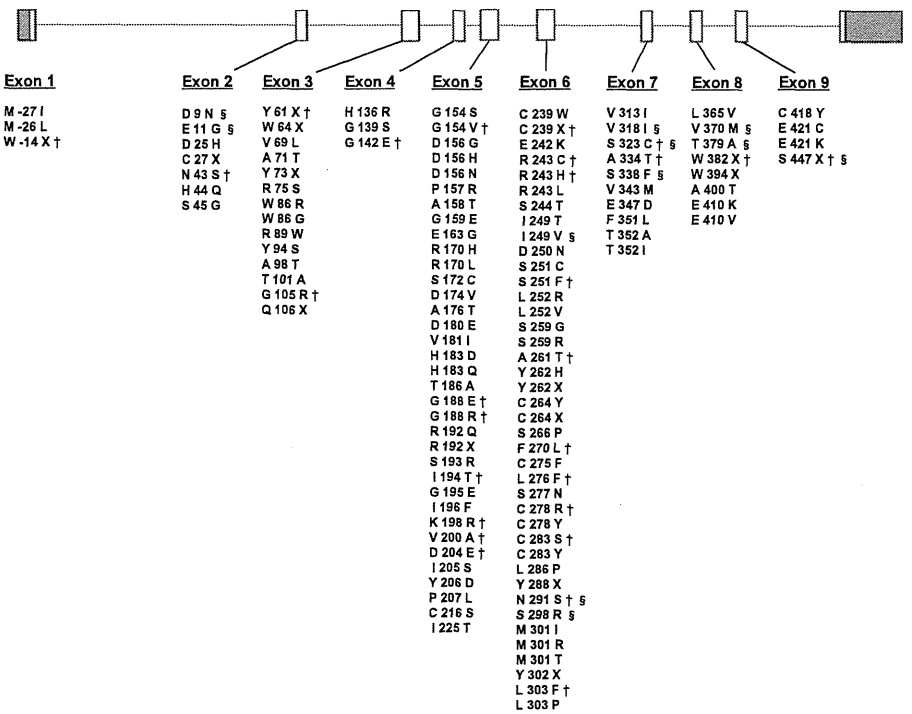

Fig. 1. Missense and nonsense mutations in the human lipoprotein lipase (LPL) gene

Each number indicates the position of affected amino acids, with +1 corresponding to the first amino acid of the mature human LPL protein.
†Mutations identified in Japanese patients with familial LPL deficiency.
§Mutations or polymorphisms not necessarily underlie LPL deficiency.

that followed-up 4 patients with LPL deficiency over 14-30 years reported that coronary angiography established atherosclerotic lesions in all patients before the age of 55 years[18], but studies on homozygotes in Japan[19, 20] both reported no advanced atherosclerotic lesion in those Japanese patients.

d) Diagnosis

Since LPL is anchored by binding with heparan sulfate on the surface of capillary endothelial cells, it appears markedly in the circulation by intravenous injection of heparin; therefore, the diagnosis is usually made by measuring plasma LPL activity and/or protein level 10 minutes after intravenous injection of heparin (10-50 U/kg). LPL protein is also present in plasma before heparin injection, but is markedly reduced or undetectable in patients with LPL null mutation (class I defect). LPL accounts for about 1/3 of the total lipase activity in plasma after heparin injection, and most of the remaining lipase activity is due to hepatic triglyceride lipase (HTGL), so diagnosis of this disorder is impossible by simple measurement of the total lipase activity. Anti-LPL and anti-HTGL antibodies are necessary for the differential measurement of LPL activities, but there is also a method to inactivate LPL using protamine sulfate or 1 M NaCl. Although this technique requires a stable synthetic substrate as well as skill and experience, measurement kits for research use are presently being marketed. Also, if either macrophages derived from peripheral blood monocytes or adipose tissue can be used as samples, differentiation from HTGL becomes unnecessary. If changes in the LPL protein level are involved, the immunological protein assay is effective and there have been a few reports on the use of ELISA in Japan[21-23], which has been adopted as a general clinical laboratory test[21]. If the LPL activity is markedly reduced, and if the concentration of apolipoprotein C-II, a critical cofactor of LPL, is normal or elevated, the diagnosis of this condition would be considered definite. Naturally, close inquiry into the familial history is often very helpful. While very rare cases with an LPL inhibitor or autoantibody are known, they can be eventually excluded by examining whether the patient's serum inhibits LPL activity in the serum of a normal control.

A diagnosis based on the LPL gene level is also widely practiced. To date, at least 163 gene mutations[2, 24, 25], including 35 in Japan alone[26], have been

Table 1. Mutations resulting from deletion/insertion or occurring at splice sites/promoter regions of the human lipoprotein lipase (LPL) gene

Deletion mutation	**Insertion mutation**	**Splice site mutation**
small deletions	*small deletions*	IVS1 ds +1 G>C
Gln(−12) Ter (del 2bp)	ins CC in 5'UTR (+14~+15)	IVS1 as −4~−2 (del 3bp)
Thr18Ter (del 11bp)	Glu35Ter (ins A)	IVS2 ds +1 G>A†
Val69Ter (del 2bp)	ins 5bp in exon 3	IVS2 as −1 G>A
Ala70Ter (del 4bp)	Lys312Ter (ins C)	IVS3 as −6 C>T†§
Lys102Ter (del 5bp)	Thr361Ter (ins A)	IVS6 as −3 C>A
Asn120Ter (del 4bp)		IVS8 ds +2 T>C†
Ser172Ter (del 1bp)	*gross insertion*	
Gly209Ter (del 1bp)	ins 2kb (exon6~IVS6)	**Promoter region mutation**
Ala221Ter (del 1bp)†		T(−93)G§
Arg243Ter (del 1bp)†	**Insertion-deletion (Indel) mutation**	G(−53)C§
Ser251Ter (del 2bp)	*small indels*	T(−39)C§
Asn291Ter (del 1bp)†	Ala70Ter (del 4bp + ins 2bp)	
Leu353Ter (del 2bp)	Thr101Ter (del 1bp + ins 6bp)	
del Ser396-Pro397 (del 6bp)	Ser193Arg + Ile194Thr (del 5bp + ins 5bp)	
gross deletions	*gross indels*	
del 54kb (5' upstream~IVS1)†	del 2.3kb inc. exon2 + ins 150bp *Alu* element†	
del 6kb (IVS2~IVS5)		
del 2.1kb (IVS7~IVS8)		
del (exon8~exon10)		

†Mutations identified in Japanese patients with familial LPL deficiency
§Mutations or polymorphisms not necessarily underlying LPL deficiency
Abbreviations: Ter, termination of codon; del, deletion; IVS, intervening sequence; UTR, untranslated region; ins, insertion; ds, donor splice site; as, acceptor splice site

identified and reported worldwide (**Fig. 1** and **Table 1**). Mutations are reportedly identified in 97% of patients, nearly 70% of which are missense mutations involving amino acid substitutions[25] that are highly concentrated in exons 5 and 6 that code for the catalytic center of LPL (**Fig. 1**); therefore, these exons should be examined first in the gene-based diagnosis of unknown mutations. Many of the amino acid substitutions cause a decrease in lipophilicity of the α-helix or β-sheet region. Other known mutations include nonsense mutations, frame-shift mutations due to insertion or deletion of a few bases, gross rearrangements due to insertion or deletion of a large DNA fragment, and splicing mutations due to mutations at splice donor or acceptor sites (**Table 1**). Since decisive mutations such as those above have been identified in most patients of European ancestry, patients who develop this disorder due to changes in the LPL gene expression levels caused by abnormality of a promoter region etc. are considered to be very rare[2]; however, since several Japanese patients are reported to be devoid of any such decisive mutations, it seems worth investigating the other region of the LPL gene in such cases.

In Japan, at least 35 mutations have been reported. In particular, as nonsense mutations in exon 3 (Y61X) and exon 8 (W382X)[27] and a single-base deletion in exon 5 (A221Ter (del 1bp))[28] have been identified in multiple families of Japanese patients, these mutations are considered to be distributed relatively widely in the LPL gene of Japanese. On the other hand, S447X, which is considered to be a gain-of-function polymorphism, has been shown to reduce TG and increase HDL-cholesterol[29].

While LPL gene mutations are relatively rare, their diagnosis is considered clinically important because of the severity of the disorders they cause. Examination of a few relatively frequent mutations has already been incorporated into routine clinical laboratory tests. Also, screening for 22 known mutations can be conducted simultaneously using the LPL gene test employing the invader method reported[30], and similar attempts are expected to make high throughput screening possible.

e) Treatment

The most problematic complication of this dis-

order is acute pancreatitis, and treatments are carried out to prevent the occurrence or progression of pancreatitis. The basic treatment is restriction of fat intake, i.e., restricting dietary fat intake to 20 g/day or less or to 15% or less of the total energy intake, to maintain the postprandial TG level at a maximum of 1,500 mg/dl or less[2]. Infants are given milk containing medium chain triglycerides (MCTs), which enter the circulation without being incorporated into chylomicrons, and defatted milk. MCTs can also be used for cooking. In the 2nd or 3rd trimesters of pregnancy, fat intake restriction up to 2 g/day has been reported not to affect neonates[2]. Acute pancreatitis is treated by fasting and low-calorie infusion, and the intravenous infusion of lipid preparations or high-calorie infusion should be avoided. This disorder barely responds to anti-hyperlipidemic drugs, but the use of fibrates should be considered in adults showing an increase also in VLDL. The effectiveness of gene therapy has been demonstrated experimentally in various animal models[31].

B) Familial Apolipoprotein C-II Deficiency

a) Concept and Definition

Apolipoprotein C-II is present primarily as a component of chylomicrons, VLDL, and HDL, and it functions on the surface of TG-rich lipoproteins as a cofactor necessary for full activation of LPL; therefore, congenital defect of this molecule causes an autosomal recessive disease that manifests marked type I or type V hyperlipoproteinemia similar to familial LPL deficiency. The first case, reported in 1978, was a 58-year-old man who had repeated episodes of acute pancreatitis accompanied by hyperchylomicronemia. The condition was not alleviated by insulin therapy for complicating diabetes, and the disease was identified incidentally as it markedly responded to transfusion performed as symptomatic therapy for anemia[32]. Similarly to LPL deficiency, consanguineous marriage is often observed in the patient's familial history, but the prevalence of this disorder is estimated to be even lower than that of LPL deficiency, and only about 20 families with this disease have been reported worldwide since it was discovered in Canada[32] and Japan[33] in the 1970s.

b) Etiology

The disease is caused by abnormality of the human apolipoprotein C-II gene and occurs in homozygotes who have inherited an abnormal apolipoprotein C-II allele from both parents (including so-called compound heterozygotes). It is inherited in an autosomal recessive pattern with penetrance of 100%. The human apolipoprotein C-II gene is located on the short arm of chromosome 19 (19q13.2), contains 4 exons, and codes for a protein with a molecular weight of 8,800, consisting of 79 amino acids[34, 35].

c) Clinical Symptoms

Since all clinical symptoms are secondary to hyperchylomicronemia, they are nearly identical to those of LPL deficiency described above; however, as the activation of LPL is partially independent of apolipoprotein C-II, clinical symptoms are often slightly milder, and, consequently, the diagnosis of the disease is often made later than LPL deficiency. As the patients tend not to be subjected to strict fat restriction from early childhood, which is more common in LPL deficiency, the incidence of acute pancreatitis has been reported to be higher in adult patients[32, 36], and hyperchylomicronemia is more often accompanied by a high VLDL level. In heterozygotes, apolipoprotein C-II is present in blood at about 50% of the normal level, and no abnormality is usually observed in the serum lipid levels, including TG.

d) Diagnosis

The diagnosis is based on demonstration of the selective absence of, or a marked decrease in, apolipoprotein C-II on clinically practical laboratory tests of serum apolipoproteins as well as clinical symptoms resembling those of LPL deficiency. The diagnosis is further supported by the presence of familial consanguinity. If LPL activity can be measured, reduced LPL activity in the patient's serum can be promptly recovered by the addition of normal human serum or purified apolipoprotein C-II. This phenomenon was also noted in the first reported Canadian patient, in whom hypertriglyceridemia was markedly improved (reduced from 1,750 to 196 mg/dl) immediately after transfusion for the treatment of anemia[32]. Another measurement method using cow's milk, which contains LPL but lacks apolipoprotein C-II, is also known.

Many families known to have this disorder have been analyzed at the gene level, and a wide variety of mutations of the apolipoprotein C-II gene have been identified, including 3 reported in Japanese patients[37-39]. Differently from LPL deficiency, apolipoprotein C-II is completely absent in many patients with this disorder due to splicing or nonsense mutation of the apolipoprotein C-II gene, but there are rare cases in which a low level of apolipoprotein C-II with a structural defect in the activation of LPL is detectable in the blood of patients. Concerning other apolipoproteins, apolipoprotein C-III and E are increased, and A-I, A-II, and B are reduced, reflecting an increase in chylomicrons and decreases in LDL and HDL.

e) Treatment

The objective of treatment for this disorder is to prevent the occurrence or exacerbation of pancreatitis, so it is treated similarly to LPL deficiency. A major difference from LPL deficiency is that serum TG can be reduced rapidly by the transfusion of normal plasma upon emergencies such as acute pancreatitis.

C) Patients Showing Inhibitors of or Autoantibodies to LPL

Families showing inhibitors of LPL in blood have been reported, and this trait is considered to be inherited in an autosomal dominant pattern[3]; therefore, in such patients, LPL activity is reportedly deficient only in blood and is normal in tissues.

Also, Kihara *et al.* noted symptoms resembling those of LPL deficiency in a young Japanese female with a history of ITP and Graves' disease, and reported the presence of an IgA autoantibody that reacts with both LPL and HTGL in her serum[4].

D) Patients with a Mutation in the Gene for GPIHBP1 or LMF1

GPIHBP1 is a capillary endothelial protein that provides a platform for LPL-mediated hydrolysis of chylomicrons, and LMF1 plays a critical role in the maturation of lipases including LPL. Recently, a few patients with mutations in these genes have also been reported to manifest type I hyperlipoproteinemia[5, 6].

Type V Hyperlipoproteinemia

According to Fredrickson's classification (WHO classification), type V hyperlipoproteinemia is defined as hyperlipoproteinemia accompanied by an increase in VLDL as well as chylomicrons. In contrast to the fact that type I hyperlipoproteinemia is mostly categorized as a condition caused by congenital abnormality of the LPL-apolipoprotein C-II system or a secondary abnormality due to marked deficiency of insulin action, type V hyperlipoproteinemia is considered to be a category that includes a wide range of pathological conditions having both congenital (genetic) and acquired (environmental) aspects and exhibiting moderate to marked hypertriglyceridemia. Indeed, upon close investigation of the patients' families, some members have been found to be hypertriglyceridemic, while many patients are associated with secondary factors such as diabetes and drinking. Since type V hyperlipoproteinemia is much more prevalent than type I, clinically encountered hyperchylomicronemia is more often type V hyperlipoproteinemia. It is difficult to accurately estimate the prevalence of type V hyperlipoproteinemia in the general population, but a survey of about 40,000 people by the Lipid Research Clinic reported the frequency of individuals with a plasma TG level of 2,000 mg/dl or higher to be about 0.018%[2]. Chylomicrons may also be observed in the blood in type Ⅲ hyperlipoproteinemia due to the inhibition of chylomicron catabolism.

Although there have been only a limited number of studies in Japan, Murase *et al.* reported the results of the evaluation of 120 Japanese with a serum TG level ≥ 1,000 mg/dl (22 type I and 98 type V patients)[7, 40]. A history of acute pancreatitis was observed in about 17% of these patients, demonstrating that hyperlipidemia is frequently complicated by pancreatitis also in Japanese, in whom the fat intake is lower than in Western people, and stressing the importance of its prevention and management. According to the cause of type I hyperlipoproteinemia, familial LPL deficiency was noted in 11, familial apolipoprotein C-II deficiency in 3, and secondary type I hyperlipoproteinemia such as diabetic lipemia in 8 (**Table 2**). Of the patients with type V hyperlipoproteinemia, the presence of underlying diseases or contributing factors such as diabetes and drinking was confirmed in about 2/3 but not in the remaining 1/3. Many of the latter patients reportedly usually show type Ⅳ hyperlipoproteinemia and have hypertriglyceridemia in the familial history.

Among congenital (genetic) abnormalities that underlie type V hyperlipoproteinemia, (1) familial combined hyperlipidemia (FCHL), which is accompanied by increased apolipoprotein B and VLDL synthesis and usually shows type Ⅱb or Ⅳ hyperlipoproteinemia, (2) monogenic familial hypertriglyceridemia accompanied by increased TG synthesis and exhibiting type Ⅳ hyperlipoproteinemia, and (3) heterozygosity of LPL gene abnormalities or abnormal expression of the LPL gene are considered important (**Fig. 2**). Such genetic abnormalities are considered to be present in a few percent of the general population and usually cause type Ⅳ hyperlipoproteinemia, some of which is considered to change to type V under the influence of environmental factors. Recently, apolipoprotein A-V was shown to strengthen the interaction between apolipoprotein C-II and LPL, suggesting that familial apolipoprotein A-V deficiency causes hyperchylomicronemia[41]. There have also been many reports that abnormalities of apolipoprotein E (E2 or E4) are involved in the pathogenesis of type V hyperlipoproteinemia[42].

While homozygous LPL deficiency can be easily diagnosed, heterozygous LPL deficiency is difficult to detect, because its phenotype may be very mild type Ⅳ hyperlipoproteinemia alone or completely asymp-

Table 2. Classification of hyperchylomicronemia according to the cause derived from data on 120 Japanese patients with a serum TG level of 1,000 mg/dL or more

		Number of patients	(males/females)
A. Hyperchylomicronemia due to abnormalities of the LPL-apolipoprotein C-II system for hydrolysis of chylomicrons			
Primary hyperchylomicronemia			
Familial LPL deficiency		11	(4/7)
Familial apolipoprotein C-II deficiency		3	(3/0)
Secondary hyperchylomicronemia			
Diabetic lipemia		6	(4/2)
Hyperlipidemia due to acromegaly		2	(0/2)
B. Type V hyperlipoproteinemia of unknown cause or underlying disorders			
Cause unknown (idiopathic)		33	(29/4)
Underlying disorders			
Complicated by diabetes (drinking: none-light)		18	(15/3)
Heavy drinking†	Non-diabetic	29	(22/7)
	Diabetic	11	(11/0)
Others§		7	

†Heavy drinking: habitual drinking of 60 g/day or more of ethanol
§2: von Gierke disease, 1: Nelson syndrome, 1: Weber-Christian disease, 1: diabetes due to L-asparaginase, 2: suspect of an LPL inhibitor
Cited from reference no. 40) Murase T: Guidelines for the Diagnosis and Treatment of Hyperlipidemia. (Bunkodo) 2005, pp 100 (in Japanese)

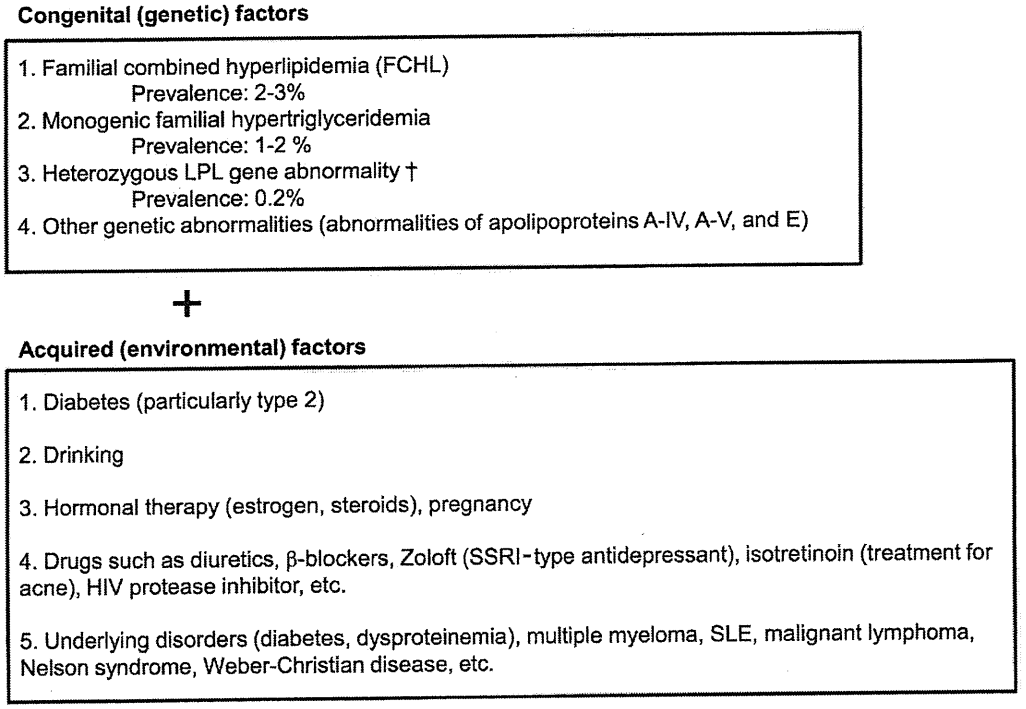

Fig. 2. Etiological factors underlying primary type V hyperlipoproteinemia

†Reported to be present in 10% of people in Western countries, but no mutation was noted in 100 Japanese subjects with a TG level of 400-1,000 mg/dl examined by Arai *et al.*[45].

Table 3. Diagnostic criteria for primary hyperchylomicronemia (draft)

Primary hyperchylomicronemia

The presence of chylomicrons in the serum confirmed after fasting for 12 hours or longer (note) is called hyperchylomicronemia, which is classified into the following 4 types.

Usually, the possibility of this disorder is high when the serum triglyceride level exceeds 1,000 mg/dl.

Note: The presence of chylomicrons can be confirmed by the appearrance of a supernatant cream layer after allowing serum to stand for 24 hours or longer at 4℃. The detection of chylomicrons by ultracentrifugation or electrophoresis (agarose or polyacrylamide gel) also contributes to the diagnosis.

1. Familial lipoprotein lipase (LPL) deficiency

(1) The absence of LPL activity in postheparin plasma, adipose tissue, or macrophages.
(2) Being a homozygote with a causative LPL gene mutation on both alleles.
(3) The presence of apolipoprotein C-II.
(4) The presence of clinical symptoms due to hyperchylomicronemia (acute pancreatitis, eruptive xanthoma, lipemia retinalis, hepatosplenomegaly).
(5) The presence of consanguinity in the familial history.
(6) A marked decrease in LPL protein mass measured by ELISA for LPL.

Definitively diagnosed if (1) or (2) is established, and provisionally diagnosed if (3) is concurrent with (4), (5), or (6).

2. Familial apolipoprotein C-II deficiency

(1) The absence of plasma (serum) apolipoprotein C-II.
(2) Being a homozygote with a causative apolipoprotein C-II gene mutation on both alleles.
(3) The appearance of activity after the addition of apolipoprotein C-II or plasma from a normal subject.
(4) The presence of clinical symptoms due to hyperchylomicronemia (acute pancreatitis, eruptive xanthoma, lipemia retinalis, hepatosplenomegaly).
(5) The presence of consanguinity in the familial history.

Definitively diagnosed if (1) or (2) is established, and provisionally diagnosed if (3) is concurrent with (4) or (5).

3. Primary type V hyperlipoproteinemia

(1) Demonstration of an increase in VLDL in addition to hyperchylomicronemia.
(2) The absence of LPL deficiency, apolipoprotein C-II deficiency, or apolipoprotein E abnormality.

Definitively diagnosed if both (1) and (2) are fulfilled.

4. Idiopathic hyperchylomicronemia

Hyperchylomicronemia not in agreement with 1, 2, or 3 above.

For example, cases suggestive of the presence of an LPL inhibitor or autoantibody have been reported. More recently, a few cases of mutations in the gene for GPIHBP1 or LMF1 have also been reported to manifest primary hyperchylomicronemia.

tomatic. In such heterozygotes, type IV-V hyperlipoproteinemia is often triggered by pregnancy, diabetes, obesity, and excessive alcohol intake. Also, there are patients with low LPL activity in families with common hyperlipidemia such as FCHL and familial hypertriglyceridemia, and the possible involvement of LPL gene abnormalities is attracting attention as a background of these disorders. Such abnormalities include abnormal LPL gene expression. Indeed, the possibility that a single nucleotide polymorphism in the promoter region, which impairs the binding of transcription factor Oct-1 and reduces transcription activity to 15% or less, is related to FCHL and ischemic heart disease has been suggested[43]. Reports from Western countries include a study in which LPL gene abnormalities were observed in 10% of patients with type V hyperlipoproteinemia[44], but Arai *et al.* found no LPL gene mutations in any of 100 Japanese subjects with a serum TG level of 400-1,000 mg/dl examined[45].

Generally, poor control of blood glucose in diabetic patients is the most frequent acquired stressor, but drinking, estrogen, steroids, pregnancy, Zoloft (selective serotonin reuptake inhibitor type antidepressant), isotretinoin (treatment for acne), diuretics, β-blockers, HIV protease inhibitors, dysproteinemia, multiple myeloma, SLE, malignant lymphoma, etc., have also been reported. Since all clinical symptoms that accompany hypertriglyceridemia are also reversible in type V hyperlipoproteinemia, fundamental treatment involves reducing the TG level. If there are strong genetic fac-

tors such as in FCHL and homozygous familial hypertriglyceridemia, strict restriction of fat intake, such as in type I hyperlipoproteinemia, may be necessary. Since acquired environmental factors are usually present in type V hyperlipoproteinemia, they must be eliminated first. Among lipid-lowering drugs, fibrates, nicotinic acid, and strong statins are indicated, but caution against possible exacerbation of the glucose tolerance is necessary in the treatment of diabetic patients with nicotinic acid. Also, as marked weight control in obese patients may induce severe hypertriglyceridemia and acute pancreatitis associated with rebound of the body weight, this risk must be considered.

Proposal of Diagnostic Criteria for Primary Hyperchylomicronemia (Draft)

Lastly, against the background described above, provisional diagnostic criteria for primary hyperchylomicronemia are presented (**Table 3**). Items related to genetic diagnosis, which has become possible, and those related to clinical symptoms and familial history have been added to the diagnostic criteria proposed by the Tarui Group[9]. Since no such diagnostic criteria or management guidelines have been established anywhere in the world, further discussion and rigorous evaluation are needed.

Conflict of Interest

Dr. Oikawa has received unrestricted grants from Daiichi-Sankyo Co. Ltd. Dr. Ishibashi has received unrestricted grants from Takeda Pharmaceutical Co. Ltd. and is an advisor of Kowa Pharmaceutical Co. Ltd. Dr. Arai has received unrestricted grants from Otsuka Pharmaceutical Co., Ltd., received honoraria from MSD, and is an advisor of Kowa Pharmaceutical Co. Ltd. Dr. Yamashita has received unrestricted grants from MSD, Otsuka Pharmaceutical Co., Ltd., Astellas Pharma Inc., and JT, collaborative research grants from Shionogi & Co., Ltd., Otsuka Pharmaceutical Co., Ltd., and National Institute of of Biomedical Innovation, honoraria for lectures from MSD, Bayer Yakuhin, Ltd., and Kowa Pharmaceutical Co.,Ltd., and is an advisory of Skylight Biotech Co. Dr. Harada-Shiba has received unrestricted grants from MSD. Dr. Eto is an advisor of MSD. The other authors declare that they have no conflict of interest.

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