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Case Report

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A Rare Case of Autoimmune-Mediated Lecithin:Cholesterol Acyltransferase Insufficiency Manifesting as the Acute Onset of Extremely Hypo-High-Density Lipoprotein-Cholesterolemia and Spontaneous Improvement: A Case Report with a Review of the Literature

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A 59-year-old Japanese woman was referred for an extremely low level of circulating high-density lipoprotein cholesterol (HDL-C). The serum HDL-C level had long been within the normal range but suddenly decreased asymptomatically to 7 mg/dL. She had no typical symptoms associated with familial lecithin, cholesterol acyltransferase deficiency (FLD), including proteinuria, anemia, and corneal opacity. The circulating level of ApoA-1 was also markedly decreased at 48 mg/dL, and the proportion of esterified cholesterol to free cholesterol was irregularly low at 26%. Whole-genome sequencing revealed no apparent pathological mutations in the *LCAT* gene. Notably, anti-LCAT antibodies were detected in the serum at 146 ± 1.7 ng/mL, resulting in her being diagnosed with acquired LCAT insufficiency (ALCATI) caused by anti-LCAT antibodies. Five years after her HDL-C levels spontaneously decreased, they increased without any identifiable cause. To our knowledge, only six cases of ALCATI caused by anti-LCAT antibodies have been reported to date. In contrast to the present case, previously reported cases of ALCATI manifested proteinuria that improved with steroid therapy. The unique clinical course in the present case highlights the heterogeneity of ALCATI, warranting further research to clarify the molecular pathophysiology of FLD and ALCATI.

Key words: **Lecithin-cholesterol acyltransferase, Hypo-high-density cholesterolemia, Autoantibody, Spontaneous improvement**

Introduction

ApoA-1, the major lipoprotein of high-density lipoprotein cholesterol (HDL-C), is synthesized in the liver and small intestine. ApoA-1 is secreted into circulation and facilitates cholesterol efflux from peripheral macrophages by interacting with ATP-binding cassette protein A1 (ABCA1), leading to the formation of preβ HDL. As preβ HDL removes free cholesterol (FC) from peripheral tissues, lecithin:cholesterol acyltransferase (LCAT) esterifies FC, allowing esterified cholesterol to migrate to the

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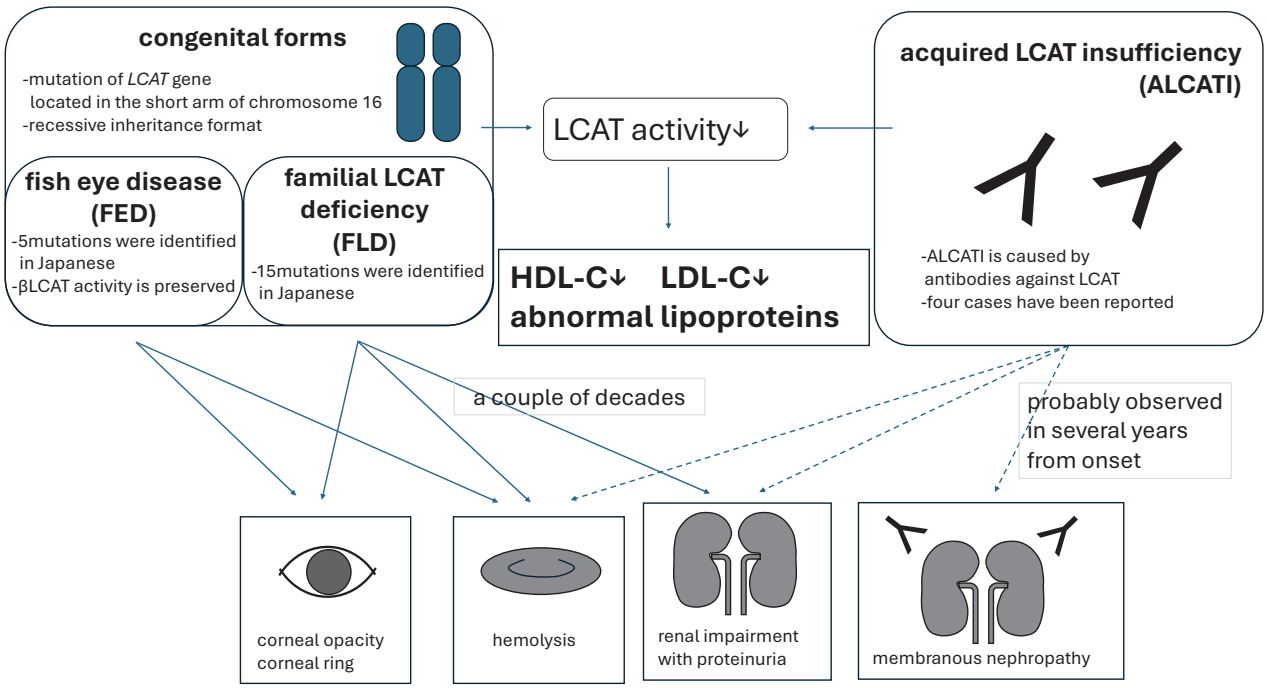

Fig. 1. Schematic illustration of phenotypic differences between FLD and ALCATI

FLD is caused by a mutation in *LCAT*, whereas ALCATI is caused by acquired antibodies against LCAT. Both conditions commonly lead to a dysfunction of LCAT activity, causing a decrease in HDL-C and LDL-C in circulation, as well as the appearance of abnormal lipoproteins. FLD often presents as corneal opacity, corneal ring, hemolysis, and renal impairment with proteinuria due to lipid deposition in the glomerular capillary walls. In contrast, ALCATI represents proteinuria associated with membranous nephropathy. Renal impairment with FLD takes a couple of decades to develop, whereas renal impairment with ALCATI occurs within a few years of onset. LCAT, lecithin:cholesterol acyltransferase; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol.

core of the particle. By repeating this process, HDL matures and transports cholesterol back into the liver[1]. This function related to HDL is mainly controlled by α-LCAT. Conversely, LCAT also plays a critical role in cholesterol esterification in low-density lipoprotein (LDL) mainly mediated by β LCAT. However, the function of β-LCAT has not been fully investigated[2].

As shown in Fig. 1, congenital and acquired forms are known to cause LCAT dysfunction. It is well known that both fish-eye disease (FED) and familial LCAT deficiency (FLD) are caused by the loss of function mutations in the *LCAT* gene[1]. To date, at least 86 cases of either homozygous or compound heterozygous mutations in *LCAT* gene have been identified in the world[3]. Patients often present with corneal opacity, corneal rings, hemolysis, and renal impairment with proteinuria due to lipid deposition[3]. In contrast, only six cases of acquired LCAT insufficiency (ALCATI) caused by antibodies against LCAT have been reported globally[4-8]. Most of them were accompanied by autoimmune diseases and nephrosis and responded well to steroid therapy.

We herein report a spontaneous improvement in ALCATI without concomitant autoimmune diseases and ALCATI-related complications.

Methods

1. Measurement of Cholesterol Levels in Serum

Cholesterol levels were measured using a homogeneous direct measurement assay during previous checkups and follow-ups, including data from Table 1 and Fig. 2. To assess lipid profiles, we performed high-performance liquid chromatography using a gel filtration column (HPLC-GFC)[9]. Serum lipoproteins from the patient were fractionated by HPLC-GFC, and levels of total cholesterol (TC), FC, triglyceride (TG), and phospholipids (PL) were quantified (Immuno-Biological Laboratories Co., Ltd., Fujioka, Japan)[10]. The lipoprotein particles were divided into 20 subclasses by diameters of lipoprotein particles using an algorithm based on the Gaussian approximation method, as follows: fraction 1, >90 nm; fraction 2, 75 nm; fraction 3, 64 nm; fraction 4, 53.6 nm; fraction 5, 44.5 nm; fraction 6, 36.8 nm; fraction 7, 31.3 nm; fraction 8, 28.6 nm; fraction 9, 25.5 nm; fraction 10, 23.0; fraction 11, 20.7 nm;

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Table 1. Lipid profiles of serum examined at initial visit, diagnosis, and after improvement

Variable	Initial visit	At diagnosis	After improvement	Reference range	Unit
HDL cholesterol	2	12	28	40-95	mg/dL
LDL cholesterol	66	37	164	65-139	mg/dL
Total cholesterol	131	176	230	120-219	mg/dL
Triglyceride	114	265	143	30-149	mg/dL
Free cholesterol	139	123	75	25-60	mg/dL
Esterified cholesterol	49	53	155	90-200	mg/dL
Cholesterol ester ratio	26	30	67	72-77	%
Phospholipid	N/A	322	254	150-280	mg/dL
Lp(a)	N/A	＜1.0	3.9	≦30.0	mg/dL
RLP-cholesterol	N/A	44.4	8.2	≦7.5	mg/dL
Apo A-I	48	63	100	126-165	mg/dL
Apo A-II	6.4	5.7	20.6	24.6-33.3	mg/dL
Apo B	63	125	138	66-101	mg/dL
Apo C-II	1.8	3.1	3.3	1.5-3.8	mg/dL
Apo C-III	4.2	5	7.3	5.4-9.0	mg/dL
Apo E	7.1	4.1	3	2.8-4.6	mg/dL
LCAT protein	N/A	0.846	2.67		μg/mL
LCAT activity※	N/A	9.3	117.0		nmol/hr/mL
Anti-LCAT antibody	N/A	163.0	211.6		ng/mL
Protenuria	negative	negative	negative	negative	

※Nanomoles of esterified cholesterol produced in per hour per milliliter
HDL, high density lipoprotein; LDL, low density lipoprotein; N/A, not applicable; Lp(a), lipoprotein(a); RLP, remnant-like particle; LCAT, lecithin:cholesterol acyltransferase.

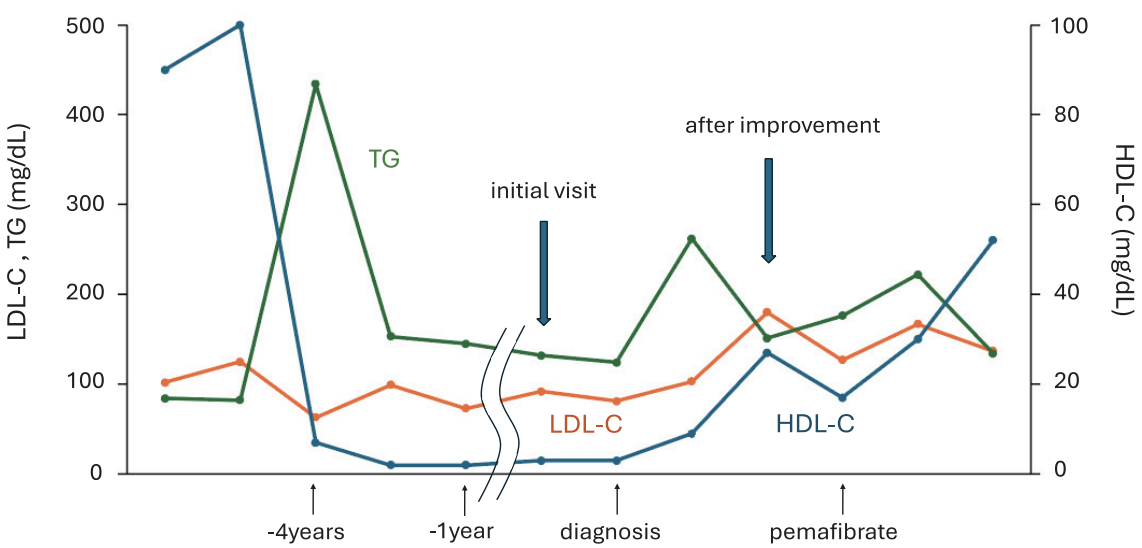

Fig. 2. Time course of serum lipid profile in the patient

The circulating level of HDL-C had been around 67-100 mg/dL until 5 years ago, when it suddenly decreased to 7 mg/dL 4 years ago without symptoms and remained low around 2-3 mg/dL thereafter. Just after the diagnosis and four years after the onset, the circulating level of HDL-C spontaneously increased without any treatments or apparent triggers. After the initiation of the administration of pemafibrate, a selective peroxisome proliferator-activated receptor α modulator (SPPARM-α), the serum lipid profile markedly improved to the normal range. The circulating level of LDL-C slightly fluctuated in synchrony with that of HDL-C. In contrast, the circulating levels of TG fluctuated inversely proportional to HDL-C and LDL-C levels. An HPLC analysis demonstrated that the serum TG levels in fractions 7 to 10, corresponding to a large LDL amount, decreased after the improvement of HDL-C (Fig. 4B), suggesting that impaired LDL hydrolysis contributed to the variation in serum TG levels. HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TG, triglyceride; HPLC, high-performance liquid chromatography.

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fraction 12, 18.6 nm; fraction 13, 16.7 nm; fraction 14, 15.0 nm; fraction 15, 13.5 nm; fraction 16, 12.1 nm; fraction 17, 10.9 nm; fraction 18, 9.8 nm; fraction 19, 8.8 nm; fraction 20, 7.6 nm.

2. Whole-Genome Sequencing (WGS)

We performed WGS on the patient using TruSeq DNA PCR-Free (Illumina, Tokyo, Japan) to prepare the WGS library, and the extracted DNA was analyzed using a NovaSeq6000 (Macrogen Japan Corp., Tokyo, Japan). We intensively searched for variants in *LCAT*, *ApoA-1*, and *ABCA1*, all of which would cause extremely low HDL-C levels in the serum.

3. Evaluating LCAT Protein

The amount of LCAT protein in the blood was measured using an enzyme-linked immunosorbent assay (ELISA)[11].

4. Assessing the LCAT Activity

To evaluate the LCAT activity in serum, we incubated serum from a patient with artificial proteoliposome substrate containing tritium-labeled free cholesterol, 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC), and ApoA-1 for 1 h at 37℃. After incubation, esterified cholesterol was quantified as previously described[10].

5. Preparation of Recombinant Human LCAT Protein (rhLCAT)

HEK293 stable clonal cells expressing the wild-type human LCAT gene were obtained. The cells were cultured in DMEM containing 10% fetal bovine serum (FBS), and the medium was replaced with SFM4 HEK293 medium (Cytiva, Marlborough, MA, USA) to prepare the LCAT-containing supernatant. Four liters of culture supernatant was filtered through a 0.45-μm filter and concentrated to 150 mL in VivaFlow 200 (MWCO=50 kDa; Sartorius AG, Göttingen, Germany). HCl (5N) was added to the concentrated supernatant to bring the pH to 5.0, followed by the addition of 1/100 volume of 1N $ZnCl_2$. NaOH (5N) was then added to bring the pH to 7.0. The sample was centrifuged at 2,300× *g* for 20 min at 4℃, and the LCAT-containing supernatant was collected. The solution was dialyzed (MWCO=50 kDa; Spectrum Labs; Repligen Corp., Rancho Dominguez, CA, USA) against 5 mM phosphate buffer and 150 mM NaCl, pH 7.4 (binding buffer). The dialyzed solution was applied to a phenyl Sepharose column (GE Healthcare Technologies Inc., Chicago, IL, USA) equilibrated with a binding buffer. The column was then washed with binding buffer, and LCAT was eluted with H_2O. A total of 1/10 volume of 10X PBS was added to the eluate. The eluate was ultrafiltered (Amicon Ultra-15 Ultracel-50K; Merck KGaA, Darmstadt, Germany), divided into small aliquots, and frozen at −80℃ until use. Protein concentrations were determined using the Bradford method (QuickStart Bradford Dye Reagent; Bio-Rad, Hercules, CA, USA).

6. Detection of Anti-LCAT Antibodies

An ELISA and immunoprecipitation followed by a Western blot analysis (IP-WB) were performed to detect anti-LCAT antibodies in the patient's serum.

6-1. Details of the ELISA

An F8 Maxisorp Nunc Immuno Module (Thermo Fisher Scientific Inc., Rochester, NY, USA) was coated overnight at 4℃ with 100 μL of 0.67 μg/mL rhLCAT in PBS. Each well was washed twice with SuperBlock T20 (TBS) Blocking Buffer (SB) (Thermo Fisher Scientific), and 200 μL of SB was added and incubated for at least 1 h at 25℃. Each well was then washed 3 times with 10% SB in TBS-T (10% SB/TBS-T), after which 100 μL of 600-fold-diluted patient serum in 10% SB/TBS-T was added at 25℃ and incubated with shaking using a microplate mixer (AS ONE Corp., Osaka, Japan) for 1.5 h.

Calibration curves were prepared using a step-diluted anti-LCAT rabbit monoclonal antibody (clone #EPR1384Y; Abcam Ltd., Cambridge, UK). After the reaction, each well was washed 4 times with TBS-T. 100 μL of 0.1 mg/mL protein G-HRP (Merck KGaA, Darmstadt, Germany) in 10% SB/TBS-T was added to each well and incubated with shaking using a microplate mixer for 1 h at 25℃. After incubation, each well was washed 5 times with TBS-T. A total of 100 μL of Dako TMB Blue Substrate-Chromogen (Agilent Technologies, Inc., Santa Clara, CA, USA) was added to each well and incubated for 10 min at 25℃. Subsequently, 50 μL of 2N H_2SO_4 was added to each well to stop the reaction. Absorbance at 450 nm was measured using a Sunrise Rainbow RC-R plate reader (Tecan Japan Co., Ltd., Kawasaki, Japan), and the anti-LCAT antibody content was analyzed using the PLATE manager V5 software program (FUJIFILM Wako Pure Chemical Corporation, Osaka, Japan).

6-2. Details of the IP-WB

A total of 3 or 10 μL of serum from each patient was mixed with 100 ng of rhLCAT protein in 500 μL of PBS containing 0.2% NP-40 (IP buffer) by rotating at 4℃ overnight. For the control reaction, 100 ng of rabbit monoclonal antibody (Abcam) was used instead of the patient's serum. Protein A-agarose (Santa Cruz

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Table 2. Profile of biochemical tests in peripheral blood at diagnosis and after improvement

Variable	At diagnosis	After improvement	Reference	Unit
WBC	5100	5700	3300-9000	/μL
RBC	380	434	380-500	x10^4/μL
Hemoglobin	12.5	14.3	11.5-15.0	g/dL
Hematocrit	39.8	42.1	34.8-45.0	%
Platelet	21.7	20.2	14.0-34.0	x10^4/μL
Reticulocyte	24	18	4-19	‰
FBG	103	94	70-109	mg/dL
HbA1c	4.1	5.0	4.6-6.2	%
AST	30	40	10-40	U/L
ALT	39	50	5-45	U/L
LDH	174	171	124-222	U/L
ALP	140	147	38-113	U/L
γ-GTP	39	44	≦30	U/L
BUN	18.6	15.6	8.0-20.0	mg/dL
Creatinine	0.5	0.52	0.47-0.79	mg/dL
eGFR	95	90.5	≧60	mL/min/1.73m^2

WBC, white blood cell; RBC, red blood cell; FBG, fasting blood glucose; HbA1c, hemoglobin A1c; AST, aspartate aminotransferase; ALT, alanine aminotrasferase; LDH, lactate dehydrogenase; ALP, alkaline phosphatase; γ-GTP, gamma-glutamyl transpeptidase; BUN, blood urea nitrogen; eGFR, estimated glomerular filtration rate.

Biotechnology Inc., Dallas, TX, USA) was washed twice with 1 mL IP buffer and resuspended in IP buffer according to the manufacturer's instructions for use in subsequent steps. A total of 20 μL of protein A-agarose were added to each reaction mixture and incubated at 4℃ for at least 2 h. The protein A-agarose bound to the antigen-IgG antibody complex was pelleted by centrifugation, washed 5 times with 1 mL of IP buffer, and eluted by heating at 100℃ in 10 μL of sample buffer (consisting of equal volumes of 2x Laemmli's sample buffer and IP buffer) containing 2.5% 2-mercaptethanol. The eluted protein samples were subjected to a Western blot analysis of LCAT using rabbit polyclonal anti-LCAT antibodies (NOVUS Biologicals, Centennial, CO, USA) as the primary antibody.

Case Presentation and Results

An asymptomatic Japanese woman of 59 years old was referred to us with incidentally identified extreme hypoHDL-cholesterolemia. Importantly, in previous health checkups, her HDL-C level in serum had long been 67-100 mg/dL until 5 years before the initial visit, at which point it suddenly decreased to 7 mg/dL and remained at approximately 2-3 mg/dL. She had no notable medical history except for a surgical operation for appendicitis around 40 years old, with no family history of dyslipidemia or autoimmune diseases. She showed no allergies to any medication or food. She was taking no medications or supplements, including anabolic androgenic steroids or lipid-lowering drugs. There were no findings of corneal opacity or corneal ring commonly observed in FLD or enlarged orange tonsils commonly observed in Tangier disease caused by mutations in the *ABCA1* gene.

According to a general blood test, hemoglobin and red blood cell (RBC) levels were within normal ranges, whereas those of reticulocytes were elevated, but hemoglobin A1c levels were decreased, suggesting slight hemolysis **(Table 2)**. Circulating levels of γ-GTP and ALP were slightly elevated, possibly because of metabolic dysfunction-associated steatotic liver disease. Data on serum lipid profiles revealed extremely low levels of HDL-C (2 mg/dL) and relatively low levels of low-density lipoprotein cholesterol (LDL-C) (66 mg/dL) **(Table 1)**. The serum ApoA-1 level decreased to 48 mg/dL (normal range: 126-165 mg/dL), ruling out both Tangier disease and congenital ApoA-1 deficiency. The proportion of esterified cholesterol in circulation was markedly decreased to 26% (normal range: 72%-77%). These findings were in accordance with the severe impairment of cholesterol esterification, particularly via serious dysfunction of LCAT. WGS revealed no pathological mutations or variants.

Based on these findings, along with her lack of a family history of FLD and a record of a normal HDL-C level in serum, we suspected ALCATI. In

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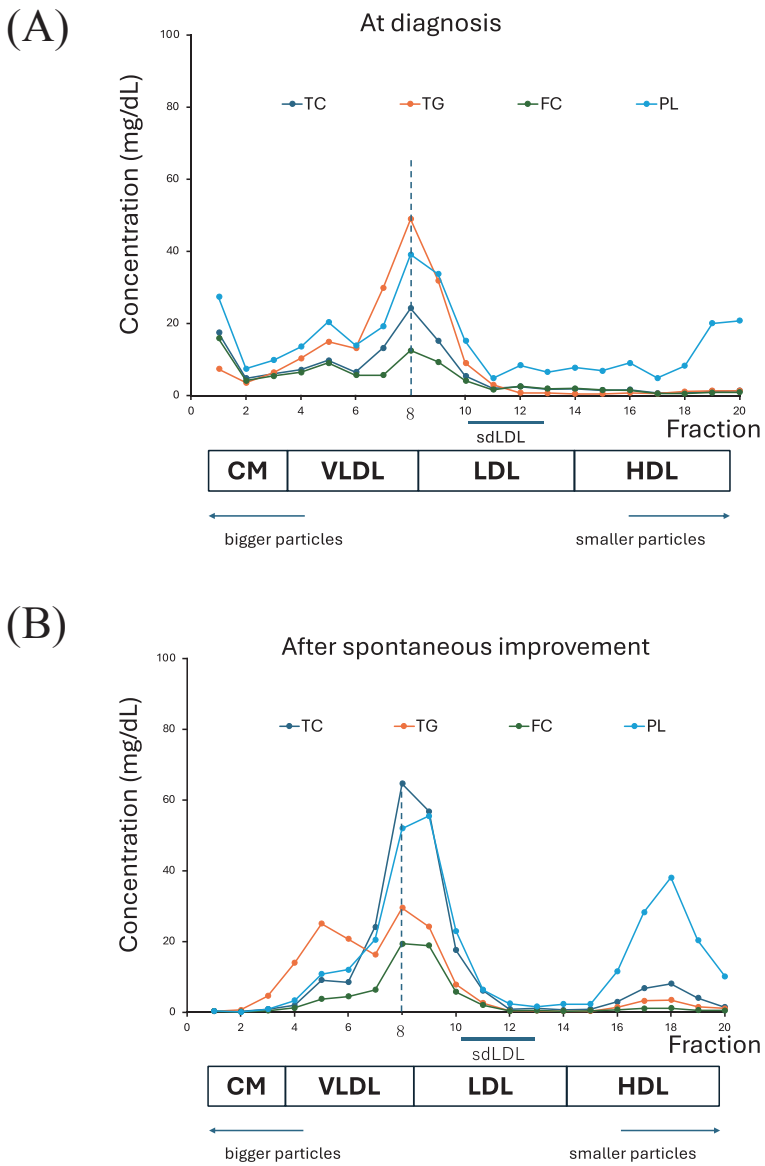

Fig. 3. Profile of HPLC-GFC analyses at the diagnosis (A) and after the spontaneous improvement (B)

At the diagnosis, TC, TG, FC, and PL were extremely low in fractions 14 to 20 related to HDL at 8.33 mg/dL, 5.61 mg/dL, 7.34 mg/dL, 77.1 mg/dL, respectively (A). After spontaneous improvement, TC, TG, FC, and PL were increased in fractions 14 to 20, at 24.6 mg/dL, 11.1 mg/dL, 4.48 mg/dL, and 112.8 mg/dL, respectively. The peaks were observed at fraction 18, which was identical in normal subjects (B). Regarding fractions related to LDL, the peaks of TC, TG, FC, and PL were observed in fraction 8, whereas these peaks were typically observed in fraction 9 in healthy subjects (A). After the spontaneous improvement, although the peaks of these lipids remained in fraction 8, the content of TC in fractions 7 to 10, corresponding to large LDL, increased from 9.35% to 28.8%, and TG in fractions 7 to 10 decreased from 42.0% to 19.8%, suggesting that the hydrolysis of LDL was improved (B). HPLC-GFC, high-performance liquid chromatography with a gel filtration column; TC, total cholesterol; TG, triglyceride; FC, free cholesterol; PL, phospholipid; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

accordance with our prediction, the concentration of LCAT protein in the blood was extremely low at 1.24 μg/mL as compared to the value of the normal pooled serum (Kohjin Bio, Sakado, Japan) of 9.28 μg/mL. Consequently, the α-LCAT activity in serum was markedly decreased at 9.3 ± 1.1 nmol/h/mL, approximately 3% from the normal pooled serum (Kohjin Bio) of 378 ± 4.9 nmol/h/mL. HPLC-GFC demonstrated that the TC in fractions 14 to 20, related to HDL-C, was significantly decreased to 8.33 mg/dL. In LDL-related fractions, the peaks of TC, TG, and FC were identified in fraction 8 **(Fig. 3A)**, whereas they were typically observed in fraction 9 in normal subjects[11]. These findings are consistent with

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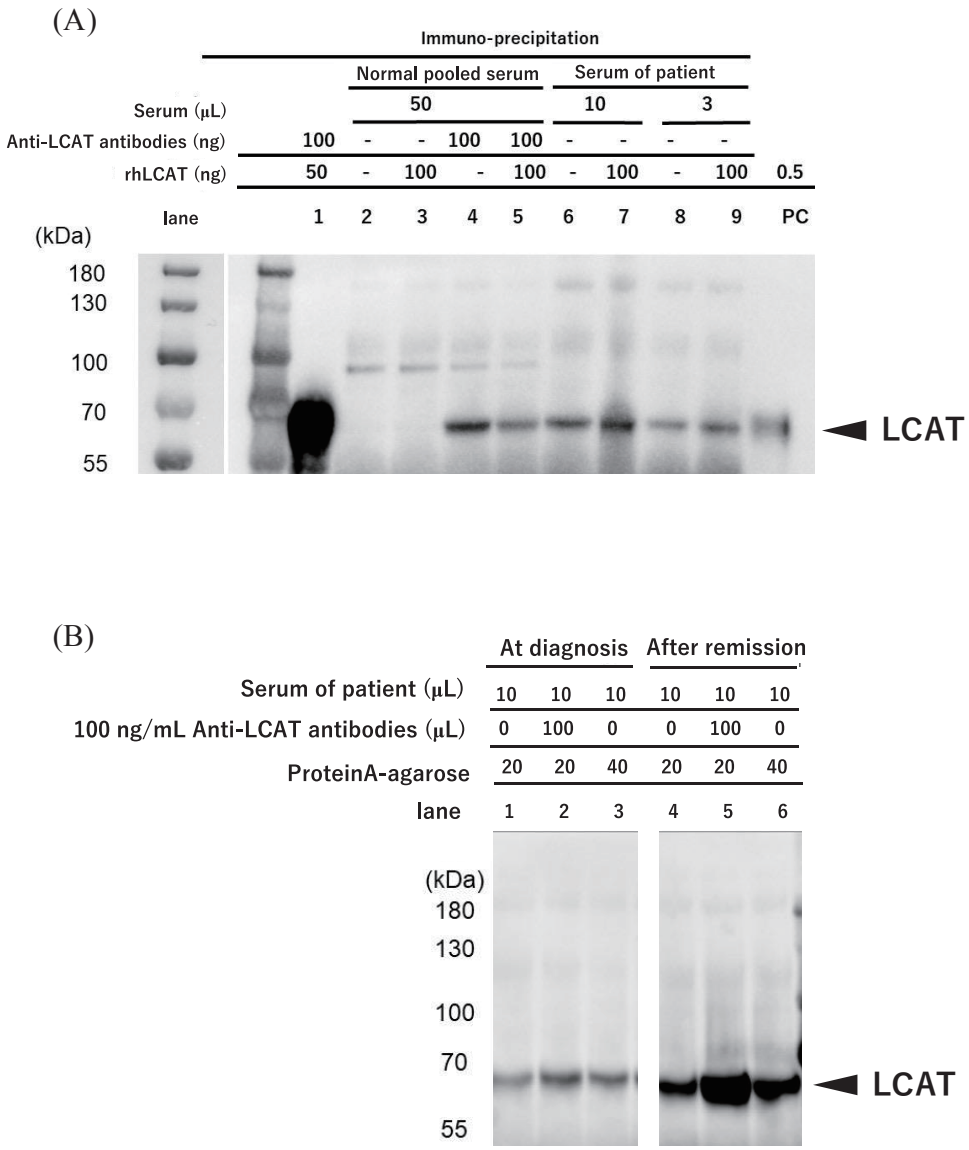

Fig. 4. Western blot analyses following immunoprecipitation: (A) Serum from the patient and a healthy subject

To confirm that the serum from the patient was duly immunoprecipitated with recombinant human LCAT (rhLCAT), we combined the serum and rhLCAT and performed immunoprecipitation and subsequent western blot analyses. we used normal pooled serum (Kohjin bio, Japan) as a negative control (lane 2, 3) and the normal pooled serum with anti-human LCAT rabbit monoclonal antibodies as a positive control (lane 4, 5). Serum from the patient was immunoprecipitated with rhLCAT (lane 7, 9). Furthermore, the patient-derived LCAT proteins were detected when serum from the patient was precipitated with magnetic beads (lane 6, 8). (B) serum from the patient with adjusted anti-LCAT antibodies and protein A-agarose beads amounts. A significantly greater amount of LCAT protein was detected after adding anti-LCAT antibodies to the serum from the patient upon improvement. Despite an increased amount of the beads, however, the amount of LCAT protein was not increased. LCAT, lecithin:cholesterol acyltransferase.

those of FLD and FED[11].

To test whether or not autoimmunity was involved in the pathophysiology of the patient, we explored anti-LCAT antibodies in serum. The relevant methods are mentioned in the Methods section. As a result, a considerable amount of anti-LCAT antibodies was detected (163.0 ng/mL) (Table 1). After the patient's serum was precipitated, patient-derived LCAT proteins were clearly detected without the addition of antibodies (lanes 6 and 8 in Fig. 4A). When rhLCAT was added to the serum of the patient, the corresponding bands were further intensified compared to the serum without rhLCAT (lane 7 vs. lane 6, and lane 9 vs. lane 8 in Fig. 4A). Therefore, we diagnosed the patient with ALCATI caused by anti-LCAT antibodies.

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Considering the extremely low HDL-C levels, we evaluated atherosclerotic lesions throughout the body, but no significant findings were observed. Carotid artery ultrasonography showed a 1.1-mm plaque. The ankle-brachial pressure index used to evaluate the presence of peripheral artery disease was 1.11 on the right and 1.05 on the left (normal range: 1.0-1.4), respectively. The brachial-ankle pulse wave velocity to evaluate arterial stiffness was 1428 cm/s on the right and 1425 cm/s on the left (abnormal range: ≥ 1800 cm/s), respectively. The flow-mediated dilatation to measure the function of the endothelium was 6.6% (normal range, >4%). Serum Lp(a), which is known to be a risk factor for atherosclerosis, was below detection.

Four years after the onset, cholesterol spontaneously increased without any treatment or apparent triggers. Compared with the initial visit and after improvement, HDL-C levels increased from 12 to 27 mg/dL, LDL-C levels were also markedly elevated from 37 to 180 mg/dL, and TG levels decreased from 265 to 151 mg/dL **(Table 1)**. Notably, the hemoglobin changed from 12.5 to 14.3 g/dL, and the percentage of reticulocytes changed from 24‰ to 18‰ **(Table 2)**. On FLD, hemolytic anemia is commonly observed due to the fragility of red blood cells caused by the abnormal lipid composition of cell membranes[3]. The findings of the present case would suggest the past coexistence of slight hemolysis with insufficient LCAT activity. HPLC-GFC analyses after the improvement confirmed that, in fractions related to HDL, TC was substantially increased from 8.33 mg/dL to 24.6 mg/dL **(Fig. 3B)**. Regarding fractions related to LDL, the peaks of TC, TG, and FC remained in fraction 8. However, the TC level of LDL fractions was markedly increased from 50.8 mg/dL to 146.8 mg/dL. The TC level in fractions 7 to 10, corresponding to large LDL, was also substantially increased from 9.35% to 28.8%. In contrast, the TG in fractions 7 to 10 markedly decreased from 42.0% to 19.8%, suggesting a considerable improvement in LDL hydrolysis **(Fig. 3B)**.

As we initially expected, both the LCAT activity and LCAT proteins in serum were substantially increased compared to the initial value, going from 9.3±1.1 nmol/h/mL to 117±2.6 nmol/h/mL and from 1.24 μg/mL to 2.67 μg/mL, respectively. In contrast, the level of anti-LCAT antibodies remained high at 211.6 ng/mL (163 ng/mL at the diagnosis) **(Table 1)**. To further explore the levels of LCAT and autoantibodies, Western blotting was performed after immunoprecipitation of the serum **(Fig. 4B)**. Immunoprecipitation of serum from the patient replenished with anti-LCAT antibodies (lane 5 in **Fig. 4B**) demonstrated a considerably higher amount of LCAT protein than that without additional anti-LCAT antibodies (lane 4 in **Fig. 4B**), and the band was not further intensified with increasing amounts of protein-A-agarose (lane 6 in **Fig. 4B**). This suggests that a considerable amount of free LCAT protein unbound to autoantibodies was present in the serum after the improvement.

The time course of lipid profiles is shown in **Fig. 2**. As TG levels remained high, pemafibrate, a selective peroxisome proliferator-activated receptor α modulator (SPPARM-α), was initiated to lower serum TG levels and increase HDL-C levels[12]. After one month of treatment, serum lipid profiles, including HDL-C, LDL-C, and TG levels, markedly improved to the normal range.

Discussion

Antibodies against ApoA-1 and lipoprotein lipase (LPL) in the circulation have been reported to cause severe dyslipidemia through autoimmune mechanisms[13, 14], and they represent a high prevalence of autoimmune diseases[14]. In contrast, to our knowledge, ALCATI, in which anti-LCAT antibodies are detected, has only been documented in six English-language studies to date **(Table 3)**. Among these patients, three had comorbidities associated with Sjögren's syndrome, sarcoidosis, or Hodgkin's lymphoma. However, the present patient had no history of chronic or autoimmune diseases. Although both ALCATI and FLD share close similarities in serum lipid profiles, both diseases differ in pathogenesis, phenotypes, and affected organs. As shown in **Table 3**, ALCATI does not exhibit corneal opacity or corneal ring, as observed in FLD. Notably, three patients presented with proteinuria, and the serum LCAT activity was below the detection limit in cases 1, 2, and 5, reflecting the severity of the disease. In contrast, the LCAT activity was measurable in the present case. In agreement with this notion, renal impairment in typical ALCATI expeditiously occurs within a few years from the onset, whereas the progression of renal damage typically takes a couple of decades in FLD, suggesting different pathogeneses[5].

In patients with FLD, abnormal lipoproteins, such as lipoprotein X (LpX) and lipoprotein 8 (Lp8), have been implicated in renal impairment[3]. LpX is abundant in PL and FC but low in TG (60%, 30%, and 2%, respectively)[3]. Conversely, Lp8 is an abnormally large LDL rich in PL, FC, and TG (41.4%, 13.2%, 45.8%, respectively)[14]. Representative pathological findings in the kidneys of patients with FLD are lipid deposition and foam cell

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Table 3. Clinical profiles of the present case and other 6 cases with ALCATI reported

	our patient	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6
age, sex	59F	67M	63F	70F	71F	70M	74M
past history	Appendicitis	Dyslipidemia (High triglyceride)	Sjogren syndrome Breast cancer	Sarcoidosis	Hodgkin lymphoma	Diabetes mellitus, Angina, Hypertension	Diabetes mellitus, Coronary artery disease, Peripheral artery disease
TC (mg/dL)	131	82	179	119	111	112	147
TG (mg/dL)	114	342	444	243	349	637	296
HDL-C (mg/dL)	2	1	3	2	5	3	1
LDL-C (mg/dL)	66	7	78	39	36	N/A	N/A
LCAT activity (nmol/hr/mL)	9.3	0	0	62.5	LCAT protein: 3.5μg/mL	0	N/A
Hb (g/dL)	12	14.4	9.4	9.6	N/A	10.7	11.3
Cre (mg/dL)	0.54	0.85	0.58	1.26	N/A	1.15	1.41
proteinuria	negative	3.2g/day	4.1g/gCr	5.6g/gCr	N/A	>3.5g/gCr	albuminuria
corneal opacification	negative	negative	negative	negative	N/A	negative	negative
carotid atherosclerosis on carotid ultrasound	1.1 mm plaque	N/A	N/A	no significant plaque	N/A	N/A	N/A
treatment	observation	PSL	PSL + AZP	diet	chemo therapy	PSL	observation
result	improved	improved	improved	improved	improved	improved	no change
reference	our patient	[4]	[5]	[6]	[7]	[8]	[8]

TC, total cholesterol; TG, triglyceride; HDL, high density lipoprotein; LDL, low density lipoprotein; LCAT; lecithin:cholesterol acyltransferase; Hb, hemoglobin; Cre, creatinine; N/A, not applicable; PSL, prednisolone; AZP, azathioprine.

infiltration in the glomeruli[3]. However, Takahashi *et al.* reported that renal pathological findings in ALCATI manifest as basement membrane duplication, spike formation, and IgG deposition along the glomerular capillary walls, suggesting a type of membranous nephropathy, all findings that are completely different from those in FLD[5]. Importantly, LCAT was also detected abundantly in glomerular capillary walls and partially colocalized with IgG, raising the possibility that LCAT could be the potential antigen responsible for membranous nephropathy in ALCATI[5].

Consistent with this notion, three patients with proteinuria (Cases 1, 2, and 5 in **Table 3**) responded well to steroid and azathioprine therapy, resulting in complete remission of proteinuria and normalization of HDL-C levels in circulation[4, 5, 8]. The patient with Hodgkin lymphoma (Case 4 in **Table 3**) underwent a series of chemotherapies comprising rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisolone, resulting in a complete cure of both lymphoma and hypoHDL-cholesterolemia[7]. Tanino *et al.* reported a case of sarcoidosis (Case 3 in **Table 3**) combined with proteinuria, that spontaneously remitted, suggesting that active sarcoidosis is related to the impairment of the LCAT function[6].

In the present case, after spontaneous improvement in HDL-C levels in circulation, anti-LCAT antibodies remained detectable **(Table 1)**. Case 5 in **Table 3** manifested proteinuria but showed excellent responsiveness to steroid therapy; anti-LCAT antibodies remained detectable after remission. Although the authors did not quantify the antibodies, they speculated that the amount would have decreased to a level sufficient to improve ALCATI[8]. In contrast, in the present case, the anti-LCAT antibody levels were comparable before and after improvement. Given these findings, along with the detection of a certain amount of free LCAT protein unbound to the autoantibodies, it is tempting to speculate that the affinity and capacity of the antibodies against LCAT proteins would have fluctuated over time. A similar phenomenon is occasionally observed in autoimmune insulin syndrome, where low-affinity and high-capacity antibodies against insulin are detected[15]. This unique property of antibodies causes fluctuations in the concentration of free insulin, resulting in brittle diabetes mellitus[15]. This phenomenon is reminiscent of that observed in the present case. However, other possibilities for the overproduction of the LCAT

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protein cannot be excluded.

In the present case, the circulating level of HDL-C further improved after initiating pemafibrate treatment. It is well known that pemafibrate increases circulating HDL-C levels by increasing ApoA-1 and expression of ABCA1 and ATP-binding cassette protein G1 (ABCG1)[13]. In fact, ApoA-1 levels increased after pemafibrate treatment. In addition, LPL activity is essential for the maturation of HDL particles by liberating FC, PL, and apoproteins from TG-rich lipoproteins. It is also known that preβHDL, which is elevated in ALCATI[4], considerably inhibits LPL activity, implying that pemafibrate increases HDL-C levels by activating LPL in ALCATI.

Also well known is that a considerably low level of HDL-C in circulation is a strong risk factor for cardiovascular events. However, there have been no reports of cardiovascular risk in ALCATI or FLD. The present case showed a 1.1-mm carotid plaque, but whether this was due to reduced HDL-C levels in circulation or simply age-related is unclear. Interestingly, Oldoni *et al.* reported that heterozygotes with *LCAT* gene mutations showed an apparently paradoxical trend of improved carotid artery thickening compared to healthy controls[16]. In contrast, Hovingh *et al.* reported that heterozygotes with *LCAT* mutations showed progressive carotid artery thickening compared with healthy controls[17]. The HPLC profile of our patient showed a peak at fraction 8 **(Fig. 3A)**, which was identical to that previously reported for both FLD and ALCATI[4, 10], whereas normal subjects showed a peak at 9. Such a shift in the peak fraction suggests that the hydrolysis of LDL was impaired, and the subsequent increase in the size of LDL was due to decreased β-LCAT activity. In fact, the concentration of fractions 10 to 13 on HPLC in our patient, which corresponds to small dense LDL (sdLDL), was apparently decreased to 11.4 mg/dL. The mean value of fractions 10–13 in normolipidemic pooled serum (14 Japanese men 22–67 years old) was reported as 25.8 mg/dL[18]. Furthermore, it is known that circulating Lp(a) levels are decreased in FLD compared to healthy subjects [19]. In the present case, decreased levels of LDL-C, sdLDL, and Lp(a) in circulation counteracted, at least partly, the atherogenicity caused by extremely low HDL-C levels.

In conclusion, we encountered a rare case of autoimmune-mediated LCAT insufficiency manifesting as an acute onset of extremely low HDL cholesterolemia. Typical ALCATI is often accompanied by autoimmune-related disorders, characterized by proteinuria associated with membranous nephropathy, and is efficiently improved with immunosuppressive therapies. However, the present case had no apparent complications and spontaneously remitted four years after the onset. Although the impact of extremely low levels of HDL-C in circulation on atherogenicity in ALCATI awaits further investigation, the present case may highlight the heterogeneity of clinical manifestations of ALCATI.

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A part of this case report was previously published as a proceeding form in a Folia Endocrinologica Japonica[20]. However, the proceeding only described the diagnostic process leading to the identification of ALCATI. On the other hand, in the current manuscript, we appended the detailed methods for evaluating the amount of anti-LCAT antibodies and enzyme activity of LCAT in circulation as well as the continuing clinical course of the patient highlighted by subsequent spontaneous improvement in hypo HDL-C but persistence of considerable amount of anti-LCAT antibodies in circulation.

Conflicts of Interest

Atsuko Tamaki, Ken Yonaha, Yohei Ishiki, Moriyuki Uehara, Yoshiro Nakayama, Ken-ichiro Honma, Rei Chinen, Tsugumi Uema, Shiki Okamoto, Junko Miyoshi, Mika Kirinashizawa, Kazuki Sato, Tsutomu Aohara, Misato Yamamoto, and Hiroaki Masuzaki have not been disclosed. Masayuki Kuroda received patent royalty and licensing fees from CellGenTech, Inc. Yoshiro Maezawa received clinical research funding from NTT Docomo, Japan. Koutaro Yokote has received honoraria from MSD K.K., Kowa Company, Ltd., Sanofi K.K., Sumitomo Pharma Co., Ltd., Daichi Sankyo Company, Limited, Taisho Pharmaceutical Co., Ltd., Mitsubishi Tanabe Pharma Corporation, Boehringer Ingelheim International GmbH., Novartis Pharma K.K., Novo Nordisk Pharma Ltd., Bayer Yakuhin, Ltd., Pfizer Japan Inc., and received clinical research funding from CellGenTech, Inc. KY also received scholarship grants from Abbot Japan LLC, Eisai Co., Ltd., Otsuka Pharmaceutical Co., Ltd., Kowa Company, Ltd., Sumitomo Pharma Co., Ltd., Taisho Pharmaceutical Co., Ltd., Takeda Pharmaceutical Company Limited, Mitsubishi Tanabe Pharma Corporation, TEIJIN PHARMA LIMITED, Eli Lily Japan K.K., Boehringer Ingelheim International GmbH, MOCHIDA PHARMACEUTICAL Co., Ltd..

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References

1) Pavanello C, Calabresi L: Genetic, biochemical, and clinical features of LCAT deficiency: update for 2020. Curr Opin Lipidol, 2020; 31: 232-237
2) Jansen M, Pfaffelhuber P, Hoffmann MM, Puetz G, Winkler K: In silico modeling of the dynamics of low density lipoprotein composition via a single plasma sample. Journal of Lipid Research, 2016; 57: 882-893
3) Kuroda M, Bujo H, Yokote K, Murano T, Yamaguchi T, Ogura M, Ikewaki K, Koseki M, Takeuchi Y, Nakatsuka A, Hori M, Matsuki K, Miida T, Yokoyama S, Wada J, Harada-Shiba M: Current Status of Familial LCAT Deficiency in Japan. J Atheroscler Thromb, 2021; 28: 679-691
4) Ishibashi R, Takemoto M, Tsurutani Y, Kuroda M, Ogawa M, Wakabayashi H, Uesugi N, Nagata M, Imai N, Hattori A, Sakamoto K, Kitamoto T, Maezawa Y, Narita I, Hiroi S, Furuta A, Miida T, Yokote K: Immune-mediated acquired lecithin-cholesterol acyltransferase deficiency: A case report and literature review. J Clin Lipidol, 2018; 12: 888-897 e882
5) Takahashi S, Hiromura K, Tsukida M, Ohishi Y, Hamatani H, Sakurai N, Sakairi T, Ikeuchi H, Kaneko Y, Maeshima A, Kuroiwa T, Yokoo H, Aoki T, Nagata M, Nojima Y: Nephrotic Syndrome Caused by Immune-Mediated Acquired LCAT Deficiency. Journal of the American Society of Nephrology, 2013; 24: 1305-1312
6) Tanino A, Okura T, Nagao T, Kukida M, Enomoto D, Miyoshi K-i, Higaki J, Kuroda M, Bujo H: A case of acquired lecithin:cholesterol acyltransferase deficiency with sarcoidosis that remitted spontaneously. CEN Case Reports, 2016; 5: 192-196
7) Simonelli S, Gianazza E, Mombelli G: Severe high-density lipoprotein deficiency associated with autoantibodies against lecithin: cholesterol acyltransferase in nonhodgkin lymphoma. Arch Intern Med, 2012; 172: 179-181
8) Komatsu T, Katsurada Y, Miyashita K, Abe S, Nishida T, Endo Y, Teramoto M, Sasaki K, Arakawa J, Sasaki M, Suzuki N, kuwata K, Imakiire T, Miyake T, Sakurada M, Matsukuma S, Hirano T, Uehara Y, Ikewaki K: Two Cases of Acquired High-Density Lipoprotein Deficiency with Immunoglobulin G4-Related Lecithin–Cholesterol Acyltransferase Autoantibody. J Atheroscler Thromb, 2023; 30: 1070-1082
9) Kuroda M, Holleboom A, Stroes E, Asada S, Aoyagi Y, Kamata K, Yamashita S, Ishibashi S, Saito Y, Bujo H: Lipoprotein subfractions highly associated with renal damage in familial lecithin:cholesterol acyltransferase deficiency. Arterioscler Thrimb Vasc Bil, 2014; 34: 1756-1762
10) Kuroda M, Aoyagi Y, Asada S, Bujo H, Tanaka S, Konno S, Tanio M, Ishii I, Machida K, Matsumoto F, Satoh K, Aso M, Saito Y: Ceiling culture-derived proliferative adipocytes are a possible delivery vehicle in enzyme replacement therapy in lecithin:cholesterol acyltransferase deficiency. The Open Gene Ther. J, 2011; 4: 1-10
11) Kobori K, Saito K, Ito S, Ito S, Kotani K, Manabe M, Kannoet T: A new enzyme-linked immunosorbent assay with two monoclonal antibodies to specific epitopes measures human lecithin-cholesterol acyltransferase. Journal of Lipid Research, 2002; 43: 325-334
12) Yamashita S, Masuda D, Matsuzawa Y: Pemafibrate, a New Selective PPARα Modulator: Drug Concept and Its Clinical Applications for Dyslipidemia and Metabolic Diseases. Current Atherosclerosis Reports, 2020; 22
13) Chistiakov DA, Orekhov AN, Bobryshev YV: ApoA1 and ApoA1-specific self-antibodies in cardiovascular disease. Laboratory Investigation, 2016; 96: 708-718
14) Kodera M, Hayakawa I, Komura K, Yanaba K, Hasegawa M, Takehara K, Sato S: Anti-lipoprotein lipase antibody in systemic sclerosis: association with elevated serum triglyceride concentrations. Rheumatol Clin Res, 2005; 32: 629-636
15) Eguchi Y, Uchigata Y, Yao K, Yokoyama H, Hirata Y, Omori Y: Longitudinal changes of serum insulin concentration and insulin antibody features in persistent insulin autoimmune syndrome (hirata's disease). Autoimmunity, 1994; 19: 279-284
16) Oldoni F, Baldassarre D, Castelnuovo S, Ossoli A, Amato M, van Capelleveen J, Hovingh GK, De Groot E, Bochem A, Simonelli S, Barbieri S, Veglia F, Franceschini G, Kuivenhoven JA, Holleboom AG, Calabresi L: Complete and Partial Lecithin:Cholesterol Acyltransferase Deficiency Is Differentially Associated With Atherosclerosis. Circulation, 2018; 138: 1000-1007
17) Hovingh GK, Hutten BA, Holleboom AG, Petersen W, Rol P, Stalenhoef A, Zwinderman AH, de Groot E, Kastelein Md JJP, Kuivenhoven JA: Compromised LCAT Function Is Associated With Increased Atherosclerosis. Circulation, 2005; 112: 879-884
18) Okazaki M, Usui S, Ishigami M, Sakai N, Nakamura T, Matsuzawa Y, Yamashita S: Identification of Unique Lipoprotein Subclasses for Visceral Obesity by Component Analysis of Cholesterol Profile in High-Performance Liquid Chromatography. Arterioscler Thromb Vasc Biol, 2005; 25: 578-584
19) Steyrer E, Durovic S, Frank S, Giessauf W, Burger A, Dieplinger H, Zechner R, Kostner GM: The role of lecithin: cholesterol acyltransferase for lipoprotein (a) assembly. Structural integrity of low density lipoproteins is a prerequisite for Lp(a) formation in human plasma. J Clin Invest, 1994; 94: 2330-2340
20) Tamaki A, Ishiki Y, Honma K, Yabiku T, Uehara M, Yamashiro K, Nakayama Y, Maezawa Y, Kuroda M, Yokote K, Masuzaki H: A Case manifesting hypo-HDL-Cholesterolemia associated with acquired LCAT insufficiency. Folia Endocrinologica Japonica [JAPANESE], 2023; 99: 70-73

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