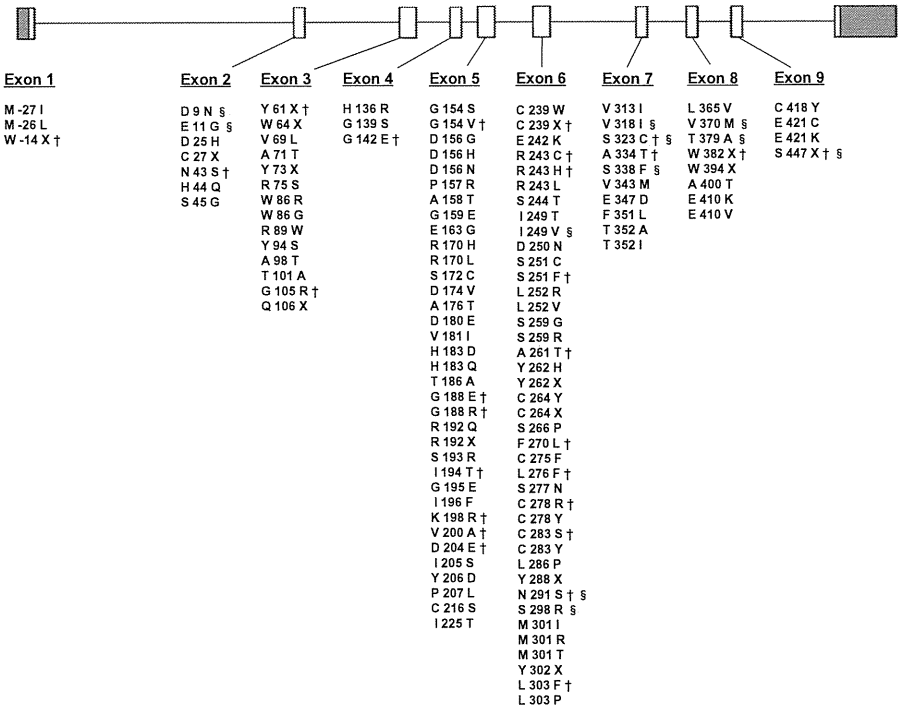

Fig. 1. Missense and nonsense mutations in the human lipoprotein lipase (LPL) gene

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)

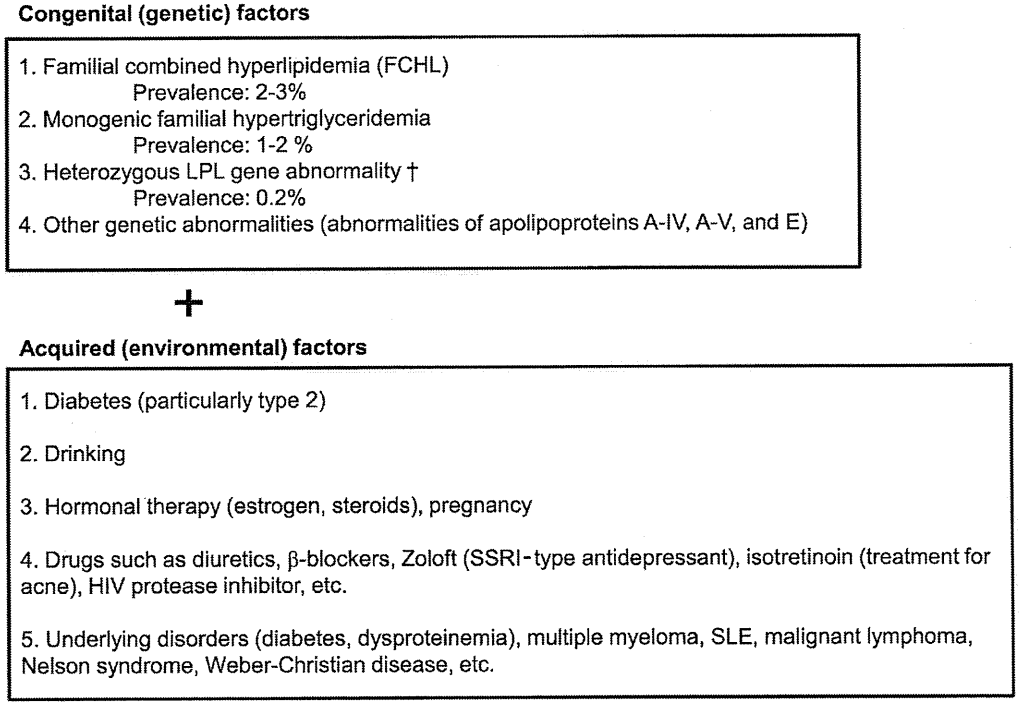

Fig. 2. Etiological factors underlying primary type V hyperlipoproteinemia

†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.

Acknowledgements

This study was supported by research grants for health sciences from the Japanese Ministry of Health, Labour and Welfare.

References

1) Fredrickson DS, Lees RS: Familial hyperlipoproteinemia. in The Metabolic Basis of Inherited Disease (ed. by Stanbury JB, Wyngaarden JB, and Fredrickson DS), 2nd ed., New York, McGraw-Hill, 1966, pp429
2) Brunzell JD, Deeb SS: Familial lipoprotein lipase deficiency, apoC-II deficiency and hepatic lipase deficiency. in The Metabolic and Molecular Bases of Inherited Disease (ed. by Scriver CR, Beaudet AL, Sly WS, Valle D, Childs B, Kinzler KW, and Vogelstein B), 8th ed., New York, McGraw-Hill, 2000, pp2789-2816
3) Brunzell JD, Miller NE, Alaupovic P, St Hilaire RJ, Wang CS, Sarson DL, Bloom SR, Lewis B: Familial chylomicronemia due to a circulating inhibitor of lipoprotein lipase activity. J Lipid Res, 1983; 24: 12-19
4) Kihara S, Matsuzawa Y, Kubo M, Nozaki S, Funahashi T, Yamashita S, Sho N, Tarui S: Autoimmune hyperchylomicronemia. N Engl J Med, 1989: 320; 1255-1259
5) Beigneux AP, Davies BSJ, Gin P, Weinstein MM, Farber E, Qiao X, Peale F, Bunting S, Walzem RL, Wong JS, Blaner WS, Ding Z-M, Melford K, Wongsiriroj N, Shu X, de Sauvage F, Ryan RO, Fong LG, Bensadoun A, Young SG: Glycosylphosphatidylinositol-anchored high-density lipoprotein-binding protein 1 plays a critical role in the lipolytic processing of chylomicrons. Cell Metab, 2007; 5: 279-291
6) Peterfy M, Ben-Zeev O, Mao HZ, Weissglas-Volkov D, Aouizerat BE, Pullinger CR, Frost PH, Kane JP, Malloy MJ, Reue K, Pajukanta P, Doolittle MH: Mutations in LMF1 cause combined lipase deficiency and severe hypertriglyceridemia. Nature Genet, 2007; 39: 1483-1487
7) Murase T, Ohkubo M: Investigation on the molecular basis of hyperchylomicronemia. Therapeutic Res, 2000; 21: 2347-2352 (in Japanese)
8) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults: Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, And Treatment of High Blood Cholesterol In Adults (Adult Treatment Panel III). JAMA, 2001; 285: 2486-2497
9) The report by the Study Group on Primary Hyperlipidemia of the Ministry of Health and Welfare (Group leader: Tarui S), 1988 (in Japanese)
10) Bürger MB, Grütz O: Uber hepatosplenomegale Lipoidose mit xanthomatosen Veranderungen der Haut und Schleimhaut. Arch Dermatol Syph, 1932; 166: 542-544
11) Havel RJ, Gordon RS Jr: Idiopathic hyperlipemia: metabolic studies in an affected family. J Clin Invest, 1960; 39: 1777-1790
12) Auwerx JH, Babirak SP, Fujimoto WY, Iverius PH, Brunzell JD: Defective enzyme protein in lipoprotein lipase deficiency. Eur J Clin Invest, 1989; 19: 433-437
13) Wion KL, Kirchgessner TG, Lusis AJ, Schotz MC, Lawn RM: Human lipoprotein lipase complementary DNA sequence. Science 1987; 235: 1638-1641

14) Senda M, Oka K, Brown WV, Qasba PK, Furuichi Y: Molecular cloning and sequence of a cDNA coding for bovine lipoprotein lipase. Proc Natl Acad Sci USA, 1987; 84: 4369-4373
15) Deeb SS, Peng RL: Structure of the human lipoprotein lipase gene. Biochemistry, 1989; 28: 4131-4135
16) Wilson DE, Emi M, Iverius PH, Hata A, Wu LL, Hillas E, Williams RR, Lalouel JM: Phenotypic expression of heterozygous lipoprotein lipase deficiency in the extended pedigree of a proband homozygous for a missense mutation. J Clin Invest 1990; 86: 735-750
17) Ma Y, Ooi TC, Liu MS, Zhang H, McPherson R, Edwards AL, Forsythe IJ, Frohlich J, Brunzell JD, Hayden MR: High frequency of mutations in the human lipoprotein lipase gene in pregnancy-induced chylomicronemia: possible association with apolipoprotein E2 isoform. J Lipid Res, 1994; 35: 1066-1075
18) Benlian P, De Gennes JL, Foubert L, Zhang H, Gagné SE, Hayden M: Premature atherosclerosis in patients with familial chylomicronemia caused by mutations in the lipoprotein lipase gene. N Engl J Med, 1996; 335: 848-854
19) Ebara T, Okubo M, Horinishi A, Adachi M, Murase T, Hirano T: No evidence of accelerated atherosclerosis in a 66-yr-old chylomicronemia patient homozygous for the nonsense mutation (Tyr61-->stop) in the lipoprotein lipase gene. Atherosclerosis, 2001; 159: 375-379
20) Kawashiri MA, Higashikata T, Mizuno M, Takata M, Katsuda S, Miwa K, Nozue T, Nohara A, Inazu A, Kobayashi J, Koizumi J, Mabuchi H: Long-term course of lipoprotein lipase (LPL) deficiency due to homozygous LPL(Arita) in a patient with recurrent pancreatitis, retained glucose tolerance, and atherosclerosis. J Clin Endocrinol Metab, 2005; 90: 6541-6544
21) Ikeda Y, Takagi A, Ohkaru Y, Nogi K, Iwanaga T, Kurooka S, Yamamoto A: A sandwich-enzyme immunoassay for the quantification of lipoprotein lipase and hepatic triglyceride lipase in human postheparin plasma using monoclonal antibodies to the corresponding enzymes. J Lipid Res, 1990; 31: 1911-1924
22) Kobayashi J, Hashimoto H, Fukamachi I, Tashiro J, Shirai K, Saito Y, Yoshida S: Lipoprotein lipase mass and activity in severe hypertriglyceridemia. Clin Chim Acta, 1993; 216: 113-123
23) Kawamura M, Gotoda T, Mori N, Shimano H, Kozaki K, Harada K, Shimada M, Inaba T, Watanabe Y, Yazaki Y, Yamada N: Establishment of enzyme-linked immunosorbent assays for lipoprotein lipase with newly developed antibodies. J Lipid Res, 1994; 35: 1688-1697
24) Gilbert B, Rouis M, Griglio S, de Lumley L, Laplaud P: Lipoprotein lipase (LPL) deficiency: a new patient homozygote for the preponderant mutation Gly188Glu in the human LPL gene and review of reported mutations: 75 % are clustered in exons 5 and 6. Ann Genet, 2001; 44: 25-32
25) http://www.ncbi.nlm.nih.gov/books/NBK1308/
26) Maruyama T, Yamashita S, Matsuzawa Y, Bujo H, Takahashi K, Saito Y, Ishibashi S, Ohashi K, Shionoiri F, Gotoda T, Yamada N, Kita T; Research Committee on Primary Hyperlipidemia of the Ministry of Health and Welfare of Japan: Mutations in Japanese subjects with primary hyperlipidemia--results from the Research Committee of the Ministry of Health and Welfare of Japan since 1996--. J Atheroscler Thromb, 2004; 11: 131-145
27) Gotoda T, Yamada N, Kawamura M, Kozaki K, Mori N, Ishibashi S, Shimano H, Takaku F, Yazaki Y, Furuichi Y, Murase T: Heterogeneous mutations in the human lipoprotein lipase gene in patients with familial lipoprotein lipase deficiency. J Clin Invest, 1991; 88: 1856-1864
28) Takagi A, Ikeda Y, Tsutsumi Z, Shoji T, Yamamoto A: Molecular studies on primary lipoprotein lipase (LPL) deficiency. One base deletion (G916) in exon 5 of LPL gene causes no detectable LPL protein due to the absence of LPL mRNA transcript. J Clin Invest, 1992; 89: 581-591
29) Kozaki K, Gotoda T, Kawamura M, Shimano H, Yazaki Y, Ouchi Y, Orimo H, Yamada N: Mutational analysis of human lipoprotein lipase by carboxy-terminal truncation. J Lipid Res, 1993; 34: 1765-1772
30) Takagi A, Ikeda Y: Measurements of lipoprotein lipase (LPL) activity and immunoreactive mass, and diagnosis of LPL gene. Nippon Rinsho, 2007; 65 (Suppl 7): 182-1890 (in Japanese)
31) Zsigmond E, Kobayashi K, Tzung KW, Li L, Fuke Y, Chan L: Adenovirus-mediated gene transfer of human lipoprotein lipase ameliorates the hyperlipidemias associated with apolipoprotein E and LDL receptor deficiencies in mice. Hum Gene Ther, 1997; 8: 1921-1933
32) Breckenridge WC, Little JA, Steiner G, Chow A, Poapst M: Hypertriglyceridemia associated with deficiency of apolipoprotein C-II. N Engl J Med, 1978; 298: 1265-1273
33) Yamamura T, Sudo H, Ishikawa K, Yamamoto A: Familial type I hyperlipoproteinemia caused by apolipoprotein C-II deficiency. Atherosclerosis, 1979; 34: 53-65
34) Jackson CL, Bruns GA, Breslow JL: Isolation and sequence of a human apolipoprotein CII cDNA clone and its use to isolate and map to human chromosome 19 the gene for apolipoprotein CII. Proc Natl Acad Sci USA, 1984; 81: 2945-2949
35) Fojo SS, Law SW, Brewer HB Jr: The human preproapolipoprotein C-II gene. Complete nucleic acid sequence and genomic organization. FEBS Lett, 1987; 213: 221-226
36) Fredrickson DS, Levy RI: Familial hyperlipoproteinemia, in The Metabolic Basis of Inherited Disease (ed. by Stanbury JB, Wyngaarden JB, and Fredrickson DS), 3rd ed. New York, McGraw-Hill, 1972, pp 545-614
37) Xiong WJ, Li WH, Posner I, Yamamura T, Yamamoto A, Gotto AM Jr, Chan L: No severe bottleneck during human evolution: evidence from two apolipoprotein C-II deficiency alleles. Am J Hum Genet, 1991; 48: 383-389
38) Inadera H, Hibino A, Kobayashi J, Kanzaki T, Shirai K, Yukawa S, Saito Y, Yoshida S: A missense mutation (Trp 26-->Arg) in exon 3 of the apolipoprotein CII gene in a patient with apolipoprotein CII deficiency (apo CII -Wakayama). Biochem Biophys Res Commun, 1993; 193: 1174-1183
39) Okubo M, Hasegawa Y, Aoyama Y, Murase T: A G+1 to C mutation in a donor splice site of intron 2 in the apolipoprotein (apo) C-II gene in a patient with apo C-II deficiency. A possible interaction between apo C-II deficiency and apo E4 in a severely hypertriglyceridemic patient.

Atherosclerosis, 1997; 130: 153-160
40) Murase T: Guidelines for the Diagnosis and Treatment of Hyperlipidemia. (Bunkodo) 2005, pp100 (in Japanese)
41) Marçais C, Verges B, Charrière S, Pruneta V, Merlin M, Billon S, Perrot L, Drai J, Sassolas A, Pennacchio LA, Fruchart-Najib J, Fruchart JC, Durlach V, Moulin P: Apoa5 Q139X truncation predisposes to late-onset hyperchylomicronemia due to lipoprotein lipase impairment. J Clin Invest, 2005; 115: 2862-2869
42) Zhang H, Reymer PW, Liu MS, Forsythe IJ, Groenemeyer BE, Frohlich J, Brunzell JD, Kastelein JJ, Hayden MR, Ma Y: Patients with apoE3 deficiency (E2/2, E3/2, and E4/2) who manifest with hyperlipidemia have increased frequency of an Asn 291-->Ser mutation in the human LPL gene. Arterioscler Thromb Vasc Biol, 1995; 15: 1695-1703
43) Yang WS, Nevin DN, Iwasaki L, Peng R, Brown BG, Brunzell JD, Deeb SS: Regulatory mutations in the human lipoprotein lipase gene in patients with familial combined hyperlipidemia and coronary artery disease. J Lipid Res, 1996; 37: 2627-2637
44) Zhang Q, Cavallero E, Hoffmann MM, Cavanna J, Kay A, Charles A, Braschi S, Marz W, Perlemuter L, Jacotot B, Galton DJ: Mutations at the lipoprotein lipase gene locus in subjects with diabetes mellitus, obesity and lipaemia. Clin Sci (Lond), 1997; 93: 335-341
45) Arai T, Tsukada T, Okubo M, Murase T, Matsumoto K: Ser477Stop mutation of the lipoprotein lipase gene occurs at a higher frequency in Japanese subjects with normal triglyceride levels than in hypertriglyceridemic patients. Atherosclerosis, 1999; 147: 417-420

Case Report

Molecular Analysis of a Novel LCAT Mutation (Gly179→Arg) Found in a Patient with Complete LCAT Deficiency

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Lecithin-cholesterol acyltransferase (LCAT) is an important enzyme involved in the esterification of cholesterol. Here, we report a novel point mutation in the LCAT gene of a 63-year-old female with characteristics of classic familial LCAT deficiency. The patient's clinical manifestations included corneal opacity, mild anemia, mild proteinuria and normal renal function. She had no sign of coronary heart disease. Her LCAT activity was extremely low. DNA sequencing revealed a point mutation in exon 5 of the LCAT gene: a G to C substitution converting Gly^{179} to an Arg, located in one of the catalytic triads of the enzyme. *In vitro* expression of recombinant LCAT proteins in HEK293 cells showed that the mutant G179R protein was present in the cell lysate, but not the culture medium. LCAT activity was barely detectable in the cell lysate or medium of the cells expressing the G179R mutant. This novel missense mutation seems to cause a complete loss of catalytic activity of LCAT, which is also defective in secretion.

J Atheroscler Thromb, 2011; 18:713-719.

Key words; LCAT, HDL, Cholesterol, Mutation

Introduction

Lecithin-cholesterol acyltransferase (LCAT) is a plasma enzyme that esterifies free cholesterol present in circulating plasma lipoproteins by catalyzing the transfer of fatty acid from the *sn*-2 position of lecithin to the 3-hydroxy group of cholesterol[1]. Mutations in the LCAT gene lead to either familial LCAT deficiency (FLD) or fish-eye disease (FED), both rare autosomal recessive disorders[2, 3]. FLD was first described in 1967 in a Norwegian family[4]. Patients with this disease often exhibit some degree of corneal opacity, anemia, proteinuria and renal disease[3]. More than 60 mutations in the LCAT gene have been identified to date (www.hgmd.cf.ac.uk/ac/index.php).

In the present study, we report a Japanese female with corneal opacity, mild anemia, mild proteinuria and normal renal function, who was carrying a novel point mutation of the LCAT gene: a G to C substitution converting Gly^{179} to an Arg, which is one of the components of the catalytic triad conserved in all animal species examined[5]. The functional significance of Gly^{179} was studied by *in vitro* expression of the mutant LCAT enzyme.

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Received: November 29, 2010
Accepted for publication: February 25, 2011

Materials and Methods

Case

The patient was a 63-year-old female diagnosed with hypocholesterolemia when hospitalized for an operation on the left meniscus 8 years ago. She was also found to have corneal opacity, anemia, and proteinuria. She also had hypothyroidism, which had been treated with levothyroxin for 2 years. She was referred to our hospital for a precise evaluation of low

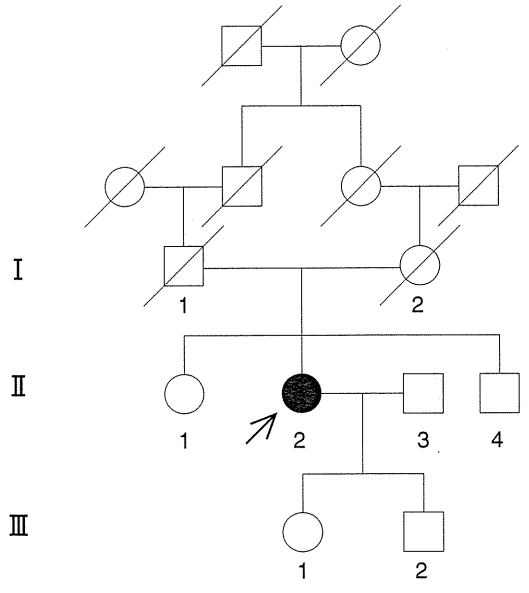

Fig. 1. Pedigree of a Japanese family with FLD.

Squares and circles indicate males and females, respectively. Slashes indicate deceased persons. Roman numerals to the left of the pedigree indicate the generation; numerals under each symbol indicate individual family members.

plasma levels of HDL-cholesterol in 2009. She had no history or signs of coronary heart disease (CHD). Her parents were first cousins (**Fig. 1**); otherwise her family history was not remarkable. Physical examination revealed no clinical abnormalities except bilateral corneal opacity and moderate loss of hearing. She had no signs of coronary heart disease.

The study adhered to the principles of the Declaration of Helsinki and was approved by our institutional ethics committee. We obtained informed consent from the patient.

Blood and Urine Collection, Plasma Lipid Analyses, and Other Clinical Parameters

After an overnight fast, blood was collected for isolation of plasma. Urine samples were collected for 24 hours for the determination of protein levels and creatinine clearance.

Total cholesterol and triglyceride concentrations were determined by enzymatic methods using commercial kits (Determiner-L TC II, Kyowa Medex and Eludia-XL TG II; Eikeukagaku Co. Ltd, Tokyo). Free cholesterol concentrations were also determined using a kit (L-type free cholesterol; Wako), as were HDL cholesterol concentrations (Determiner-L HDL-C; Kyowa Medex), plasma levels of apolipoproteins (Apolipoproteins auto N; Sekisui Medical) and cholesterol ester transfer protein concentrations (CETP ELISA-DAIICHI; Sekisui Medical). Fifteen min after iv injection of 30 U/kg heparin, plasma was collected for the measurement of lipoprotein lipase protein concentration (LPL ELISA-DAIICHI; Sekisui Medical)[6].

LCAT Activity Assay

LCAT activity in plasma was measured using a proteoliposome substrate in a LCAT assay kit (Anasorb® LCAT; Sekisui). When intracellular extracts and cell culture medium were used as the LCAT source, proteoliposomes were prepared by 30-min incubation of apoA-I (Sigma) with the proteoliposomes provided by the kit at a molar ratio to cholesterol of 0.8:12.5 at 37℃[7].

DNA Isolation

Genetic DNA was isolated from 2 mL peripheral blood using a QIAamp® DNA Blood Midi Kit (Qiagen) and stored at 4℃.

DNA Amplification by PCR

Oligonucleotide primers were synthesized by Oligo® Sigma Genosys. The primers used in the PCR of genomic DNA and the PCR program have been reported previously[8].

DNA Sequence Analysis

PCR-amplified DNA was purified with a QIAquick® Gel Extraction Kit (Qiagen) and re-amplified by PCR using an unequal ratio (10/1) of the same primers with the BigDye Terminator V1.1 cycle sequencing kit (Applied Biosystems). Single-stranded DNA (20 μL) from the second PCR was purified and precipitated in 99% ethanol, resolved in 25 μL Hi-Di Formamide and sequenced by a 3100 Genetic Analyzer (Applied Biosystems).

Apoe Genotyping

Restriction isotyping of common *APOE* isoforms was carried out by gene amplification and cleavage with HhaI as previously described[9].

Site-Directed Mutagenesis and Construction of LCAT cDNA Expression Vectors

Total RNA was isolated from HepG2 cells using TRIzol® Reagent (Invitrogen). The RNA was reverse transcribed with a High Capacity cDNA Reverse Transcription Kit (Applied Biosystems), and amplified by PCR using the primers 5'ATGGGGCCGCCCGGCTCC3' and 5' AGGAGGCGGGGGCTCTGG3'

Table 1. Clinical and laboratory data of the patient and her family members

ID		Ⅱ-1	Ⅱ-2 (proband)	Ⅱ-4	Ⅲ-2
Sex		F	F	M	M
Age		68	63	56	39
BMI		20.6	22.0	24.9	24.9
CBC	RBC ($\times 10^4$)	380	358		507
	Hb (g/dL)	11.2	10.3	14.7	14.9
	Hct (%)	33.7	30.0	43.9	46.4
	WBC		5,100		6,300
	Plt ($\times 10^4$)		14.9		23.6
Chemistry	Creatinine (mg/dL)	0.52	0.64	0.73	0.8
Urine	Protein	−	+	−	−
Lipids	Total cholesterol (mg/dL)	155	76	225	169
	Triglyceride (mg/dL)	50	89	118	195
	HDL-cholesterol (mg/dL)	36	6	34	31

The data of Ⅱ-1, Ⅱ-4 and Ⅲ-2 were adopted from the results of regular health checks.
IDs are indicated in Fig. 1.

to obtain whole cDNA of LCAT without a stop codon. This PCR product was then subcloned into pCR2.1®-TOPO (Invitrogen) by TA cloning, and the single-stranded DNA of the coding regions was confirmed by sequencing. The T vector and expression vector pcDNA3.1/*myc*-His A (Invitrogen) were prepared by double digestion with HindIII and XhoI, purified by gel extraction using a QIAquick Gel Extraction Kit (Qiagen) and ligated by T4 DNA ligase using a DNA Ligation Kit, Ver.2.1 (TaKaRa). After confirmation by sequencing, an expression plasmid for LCAT cDNA with a C-terminal myc epitope and the polyhistidine tag was successfully constructed. This plasmid was modified using a QuickChange® Site-Directed Mutagenesis kit (Stratagene) with the primers 5'CCTGTCTTCCTCATTCGCCACAGCCTC-GGCTG3' and 5'CAGCCGAGGCTGTGGCGAAT-GAGGAAGACAGG3' by substituting one base, GGC (Gly of the wild type) for CGC (Arg of the mutant), at codon 179 in exon 5. Also, the mutant plasmid was confirmed by sequencing.

Transient Expression of the LCAT cDNA *in vitro*

HEK293 cells (4×10^5) were suspended in 5 mL Dulbecco's modified Eagle's medium (DMEM; Sigma) supplemented with 10% fetal calf serum (FCS) and incubated in a 60-mm tissue culture dish at 37℃ under an atmosphere of 5% CO_2 and 95% air until 80% confluent. The plasmids (5 μg) were introduced into HEK293 cells in triplicate using the SuperFect® Transfection Reagent (Qiagen). The cell culture medium was changed to FCS-free DMEM after 24 hours and cells were harvested after another 72 hours. Intracellular proteins were extracted from the cells by centrifugation and resuspension of the cell pellet in 0.2 mL RIPA buffer (1% NP-40, 150 mM NaCl, 50 mM Tris-HCl pH7.4, 0.1% SDS, 0.1% sodiumdeoxycholate) with a cocktail of proteinase inhibitors (1 mM PMSF, 2 μM leupeptin and 0.08 μM aprotinin). The medium was concentrated by Amicon Ultra-30 (Milipore) by about 10-fold. The protein concentration was determined using a BCA kit (Pierce). Aliquots of the medium and the intracellular extracts were kept at −80℃ until Western blotting and LCAT assays were performed.

Western Blot Analysis

5×SDS sample buffer (Sigma) was added to 20 μg cell lysate or 20 μL concentrated medium, and boiled for 5 min. After centrifugation, the supernatant was subjected to 10% SDS-PAGE, which was run at 10 mV for 3 hours. Western blot analysis was performed with an anti-myc antibody (Invitrogen). Horseradish peroxidase-conjugated secondary antibody and the ECL Western blotting detection reagents system (Amersham) were used for detection. The signal was visualized with X-ray film (Roche Diagnostics).

Results

Clinical Data

Table 1 summarizes the clinical and laboratory data of the patient and her family members. The

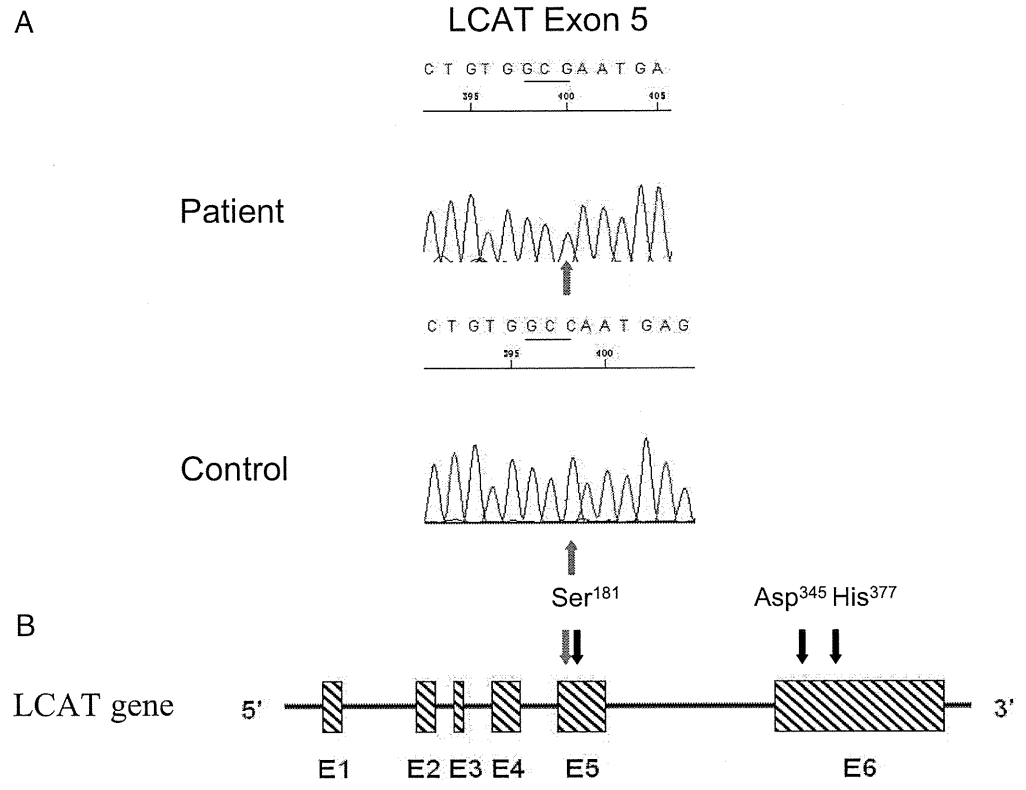

Fig. 2. Sequence result of the patient.

(A) Direct sequencing showed that this patient was homozygous for a novel mutation in exon 5 of the LCAT gene. A nucleotide transition in exon 5 (c.607 G>C) was found which results in an amino acid change at codon 179 from glycine (GGC) to arginine (CGC). (B) The whole structure of the LCAT gene, in which the position of the catalytic triad is marked.

patient had mild anemia with poikilocytosis, such as target cells in blood smears and mild proteinuria (0.23 g/24 h). No abnormalities were detected in liver, renal and thyroid function tests or in plasma electrolytes. Fasting blood glucose was 100 mg/dL. Plasma haptoglobin level (52 mg/dL) was low, indicating the presence of hemolysis. Total cholesterol and HDL cholesterol levels were markedly reduced. The ratio of cholesterol ester to total cholesterol (0.105) was also markedly decreased. Plasma levels of apo A-I, A-II, B, C-II and C-III were low (33, 3.5, 40, 1.4 and 2.6 mg/dL, respectively). Apo E level was 5.9 mg/d. Lipoprotein (a) was undetectable. Lipoprotein X was positive. RLP-C (33.6 mg/dL) was increased. LCAT activity (<50 nmol/mL/hr) was almost undetectable. Post-heparin lipoprotein lipase mass (85 ng/mL) was decreased. Plasma CETP concentration (2.5 ng/mL) was normal. Creatinine clearance was 113 mL/min. Titers of anti-thyroglobulin and anti-thyroid peroxidase antibodies were 3.6 and <0.3 U/L, respectively. Plasma Ig G level was mildly elevated (1,816 mg/dL). Anti-nuclear antibody was negative. The patient was a carrier of APOE $\varepsilon 3/\varepsilon 3$ genotype.

Plasma HDL-C levels of the family members (II-1, II-4 and III-1) were slightly reduced (**Table 1**).

DNA Sequence Analysis

Direct sequencing showed that this patient was homozygous for a novel mutation in exon 5 of the LCAT gene (**Fig. 2**). A nucleotide transition in exon 5 (c.607 G>C) was found which results in an amino acid change at codon 179 from glycine (GGC) to arginine (CGC).

Western Blot Analysis

Immunoblotting of the lysate of transfected cells expressing the wild-type or mutant LCAT showed a single band with a molecular weight of about 52 kDa. Although the medium from the cells expressing wild-type LCAT showed a single band of about 67 kDa, corresponding to the fully glycosylated form of LCAT[10], the mutant LCAT (LCAT G179R) was not detected in the medium (**Fig. 3A**).

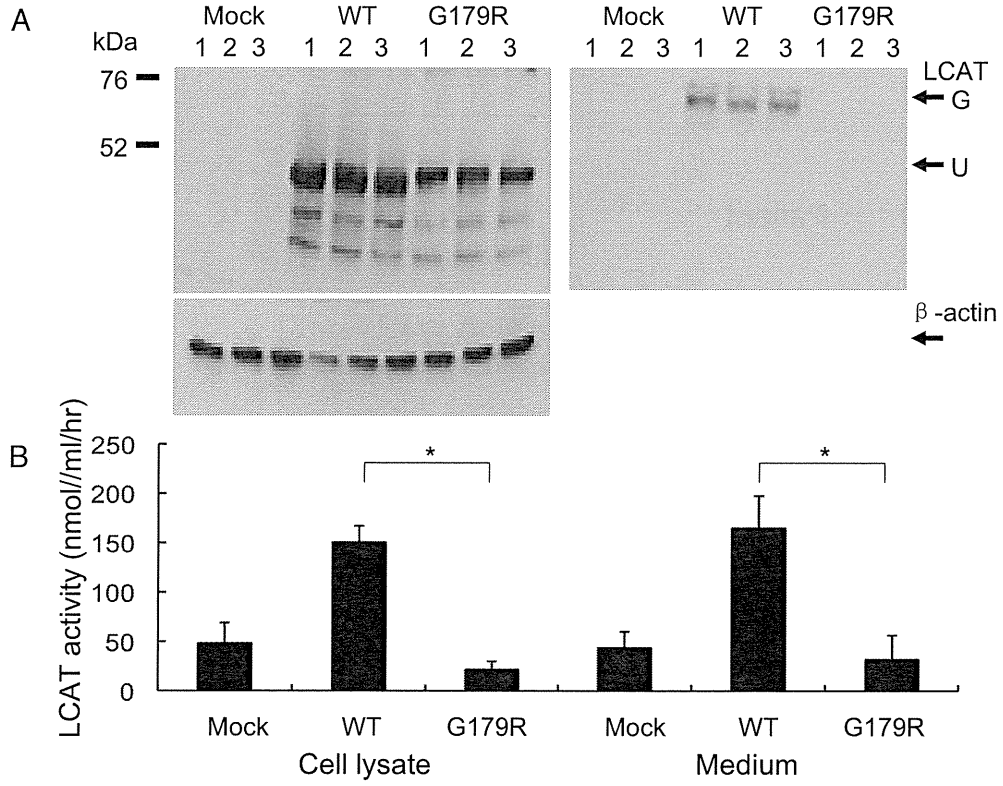

Fig. 3. Functional analyses of LCAT expressed in vitro.

(A) Western blot analysis of LCAT protein. G denotes glycosylated form; U denotes unglycosylated form. (B) LCAT activities. Wild-type (WT) or mutant LCAT G179R was expressed in HEK293 cells in triplicates (Lanes 1-3). Cell lysate or medium was used for Western blot analysis or duplicate measurements of LCAT activity. Data are the means ± S.D. $^{*}p < 0.05$.

LCAT Activity

LCAT activity was detected in both the cell lysate and medium of the cells expressing wild-type LCAT, while it was barely detectable in either the lysate or medium of cells expressing the mutant LCAT (**Fig. 3B**).

Discussion

Since 1967, over 60 variations in the LCAT gene have been described[3, 11-14], which are classified into two rare autosomal recessive disorders: FLD and FED, characterized by a low HDL-C concentration, corneal opacity, hemolytic anemia and renal involvement[3].

In this case, the deficiency of plasma LCAT activity, which was associated with corneal opacity, mild hemolytic anemia and a very low HDL-C level, is supportive of the diagnosis of classical LCAT deficiency. Direct sequencing of the LCAT gene revealed a novel point mutation in exon 5 (c.607 G>C), which is predicted to result in an amino acid change at codon 179 from glycine (GGC) to arginine (CGC) (**Fig. 2**). This mutation is located in the catalytic triad of the enzyme, formed by Ser^{181}, Asp^{345} and His^{377} [15]. G^{179} is part of the consensus sequence GXSXG, the signature motif of the α/β hydrolase family[16], and also part of the conserved strand of β-pleated sheet 5, which is important for the proper folding of the enzyme[5]. Mutations of the other two key amino acids of this motif (S^{181} and G^{183}) have been reported, all of which can disrupt the LCAT activity[3, 17].

Since other FLD mutants are also located at positions that are strictly conserved[5], we believe that the homozygous mutation G179R in LCAT accounts for the clinical manifestations in this case of FLD.

As predicted from the structure, LCAT activity was barely detectable in the cell lysate or in the medium of cultured cells transiently expressing the mutant LCAT (G179R) (**Fig. 3**). Furthermore, the mutant protein itself was not detected in the culture medium, although it was detectable in the cells, suggesting that LCAT G179R is defective in secretion. Taramelli *et al.* reported a similar non-secreting LCAT mutant, R147W, in a patient who lacked LCAT activ-

ity in plasma[18]. The R147W mutation substitutes a bulky tryptophan for arginine, thereby presumably inhibiting the formation of a salt bridge[5].

Three of the family members exhibited a mild decrease in plasma HDL- cholesterol levels (**Table 1**). Although we have not measured LCAT activity or genotyped for the mutation in these family members, they may be obligate heterozygotes for the mutation, being responsible for the relatively low plasma HDL-cholesterol levels[19]. Renal disease is the major cause of morbidity and mortality in FLD patients[20], but the clinical manifestations of FLD patients vary even among family members carrying same mutations[17, 20]. The GFR of the patient was nearly normal, despite the presence of mild proteinuria. The progression of nephropathy may have been delayed in this patient because she was a vegetarian.

Lipoprotein (a) in the patient was almost undetectable. Although it is possible that the low lipoprotein (a) level was caused by apo (a) genotype, absence of LCAT activity may have caused a failure to produce LDL particles of the right morphology and chemical composition to allow lipoprotein (a) formation[21].

Baass A *et al.* have recently reported that the *APOE* genotype significantly influences the phenotypic expression of familial LCAT deficiency[19]. Consistent with their observations, the patient, who had $\varepsilon 3/\varepsilon 3$ genotype, had a relatively low plasma level of triglyceride.

In summary, we report a novel point mutation, G179R, in the LCAT gene of a homozygous FLD patient. This mutation is located in the catalytic triad of the enzyme and may affect the secretion and activity of LCAT protein.

Acknowledgements

We are grateful to Dr. Masayuki Kuroda and Prof. Hideaki Bujo at Chiba University for confirmation of the absence of LCAT activity in the plasma. This work was supported by a Grant-in-Aid for Scientific Research from the Ministry of Health, Labour and Welfare, the Program for Promotion of Fundamental Studies in Health Sciences of the National Institute of Biomedical Innovation (NIBIO), and JKA through its promotion funds from KEIRIN RACE.

References

1) Glomset JA: The plasma lecithins:cholesterol acyltransferase reaction. J Lipid Res, 1968; 9: 155-167
2) Kuivenhoven JA, Pritchard H, Hill J, Frohlich J, Assmann G, Kastelein J: The molecular pathology of lecithin: cholesterol acyltransferase (LCAT) deficiency syndromes. J Lipid Res, 1997; 38: 191-205
3) Santamarina-Fojo S, Hoeg JM, Assmann G, Brewer JHB: Lecithin cholesterol acyltransferase deficiency and fish eye disease, In: Scriver CR, Beaudet AL, Sly WS, Valle D: (eds), The metabolic and molecular bases of inherited disease, New York, McGraw-Hill, 2001: 2817-2833
4) Norum KR, Gjone E: Familial serum-cholesterol esterification failure. A new inborn error of metabolism. Biochim Biophys Acta, 1967; 144: 698-700
5) Peelman F, Verschelde JL, Vanloo B, Ampe C, Labeur C, Tavernier J, Vandekerckhove J, Rosseneu M: Effects of natural mutations in lecithin: cholesterol acyltransferase on the enzyme structure and activity. J Lipid Res, 1999; 40: 59-69
6) Kobayashi J, Hashimoto H, Fukamachi I, Tashiro J, Shirai K, Saito Y, Yoshida S: Lipoprotein lipase mass and activity in severe hypertriglyceridemia. Clin Chim Acta, 1993; 216: 113-123
7) Chen CH, Albers JJ: Characterization of proteoliposomes containing apoprotein A-I: a new substrate for the measurement of lecithin: cholesterol acyltransferase activity. J Lipid Res, 1982; 23: 680-691
8) Argyropoulos G, Jenkins A, Klein RL, Lyons T, Wagenhorst B, St Armand J, Marcovina SM, Albers JJ, Pritchard PH, Garvey WT: Transmission of two novel mutations in a pedigree with familial lecithin: cholesterol acyltransferase deficiency: structure-function relationships and studies in a compound heterozygous proband. J Lipid Res, 1998; 39: 1870-1876
9) Hixson JE, Vernier DT: Restriction isotyping of human apolipoprotein E by gene amplification and cleavage with HhaI. J Lipid Res, 1990; 31: 545-548
10) Chisholm JW, Gebre AK, Parks JS: Characterization of C-terminal histidine-tagged human recombinant lecithin: cholesterol acyltransferase. J Lipid Res, 1999; 40: 1512-1519
11) Bujo H, Kusunoki J, Ogasawara M, Yamamoto T, Ohta Y, Shimada T, Saito Y, Yoshida S: Molecular defect in familial lecithin: cholesterol acyltransferase (LCAT) deficiency: a single nucleotide insertion in LCAT gene causes a complete deficient type of the disease. Biochem Biophys Res Commun, 1991; 181: 933-940
12) Gotoda T, Yamada N, Murase T, Sakuma M, Murayama N, Shimano H, Kozaki K, Albers JJ, Yazaki Y, Akanuma Y: Differential phenotypic expression by three mutant alleles in familial lecithin: cholesterol acyltransferase deficiency. Lancet, 1991; 338: 778-781
13) Calabresi L, Pisciotta L, Costantin A, Frigerio I, Eberini I, Alessandrini P, Arca M, Bon GB, Boscutti G, Busnach G, Frasca G, Gesualdo L, Gigante M, Lupattelli G, Montali A, Pizzolitto S, Rabbone I, Rolleri M, Ruotolo G, Sampietro T, Sessa A, Vaudo G, Cantafora A, Veglia F, Calandra S, Bertolini S, Franceschini G: The molecular basis of lecithin: cholesterol acyltransferase deficiency syndromes: a comprehensive study of molecular and biochemical findings in 13 unrelated Italian families. Arterioscler Thromb Vasc Biol, 2005; 25: 1972-1978
14) Hirashio S, Izumi K, Ueno T, Arakawa T, Naito T, Taguchi T, Yorioka N: Point mutation (C to T) of the LCAT

gene resulting in A140C substitution. J Atheroscler Thromb, 2010; 17: 1297-1301
15) Peelman F, Goethals M, Vanloo B, Labeur C, Brasseur R, Vandekerckhove J, Rosseneu M: Structural and functional properties of the 154-171 wild-type and variant peptides of human lecithin-cholesterol acyltransferase. Eur J Biochem, 1997; 249: 708-715
16) Ollis DL, Cheah E, Cygler M, Dijkstra B, Frolow F, Franken SM, Harel M, Remington SJ, Silman I, Schrag J, Sussman JL, Verschueren KHG, Goldman A: The alpha/beta hydrolase fold. Protein Eng, 1992; 5: 197-211
17) Frasca GM, Soverini L, Tampieri E, Franceschini G, Calabresi L, Pisciotta L, Preda P, Vangelista A, Stefoni S, Bertolini S: A 33-year-old man with nephrotic syndrome and lecithin-cholesterol acyltransferase (LCAT) deficiency. Description of two new mutations in the LCAT gene. Nephrol Dial Transplant, 2004; 19: 1622-1624
18) Taramelli R, Pontoglio M, Candiani G, Ottolenghi S, Dieplinger H, Catapano A, Albers J, Vergani C, McLean J: Lecithin cholesterol acyl transferase deficiency: molecular analysis of a mutated allele. Hum Genet, 1990; 85: 195-199
19) Baass A, Wassef H, Tremblay M, Bernier L, Dufour R, Davignon J: Characterization of a new LCAT mutation causing familial LCAT deficiency (FLD) and the role of APOE as a modifier gene of the FLD phenotype. Atherosclerosis, 2009; 207: 452-457
20) Borysiewicz LK, Soutar AK, Evans DJ, Thompson GR, Rees AJ: Renal failure in familial lecithin: cholesterol acyltransferase deficiency. Q J Med, 1982; 51: 411-426
21) 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

家族性高コレステロール血症

ステートメント

1. 家族性高コレステロール血症（Familial Hypercholesterolemia、 以下 FH）は、高 LDL コレステロール血症、若年性冠動脈疾患、腱・皮膚黄色腫を 3 主徴とする常染色体優性遺伝性疾患であり、きわめて冠動脈疾患のリスクが高く、早期診断、厳格な治療が推奨される（推奨レベルⅠ、エビデンス B）
2. FH ヘテロ接合体の診断は①LDL コレステロール 180mg/dL 以上、②腱・皮膚黄色腫、③2 親等以内の血族の FH あるいは若年性冠動脈疾患の家族歴のうち、2 つ以上の項目を満たすこととする（推奨レベルⅠ、エビデンス C）
3. FH ヘテロ接合体の治療は、スタチンを中心とした厳格な脂質管理を行うことが勧められる（推奨レベルⅠ、エビデンス B）
4. FH ヘテロ接合体の診断後は、個人のリスクを考慮した冠動脈疾患のスクリーニング検査を定期的に施行することが推奨される（推奨レベルⅠ、エビデンス C）
5. ホモ接合体および薬物療法抵抗性の重症ヘテロ接合体に対して、LDL アフェレシス治療を施行することが推奨される（推奨レベルⅠ、エビデンス B）
6. ホモ接合体および薬物療法抵抗性、小児、妊娠中及び挙児希望のヘテロ接合体は専門医へ紹介することが推奨される（推奨レベルⅠ）

① FH ヘテロ接合体

Ⅰ. FH の病態と臨床像、遺伝子変異

家族性高コレステロール血症（Familial Hypercholesterolemia、 以下 FH）は、1）高 LDL コレステロール血症、2）若年性冠動脈疾患、3）腱・皮膚黄色腫を 3 主徴とする常染色体優性遺伝性疾患である。FH は単独で極めて冠動脈疾患のリスクが高い病態であり、未治療の男性で 30〜50 歳、女性で 50〜70 歳の間に心筋梗塞、狭心症などの冠動脈疾患を発症することが多く（1）、早期診断を行い、適切な治療を行うことが、若年死を予防することにつながる。300〜500 人に 1 名の割合で FH ヘテロ接合体が存在し、日本全体では約 30 万人の患者がいると推定されるため、実地医家が遭遇する最も高頻度の遺伝疾患の一つである。

Ⅰ-1. 病態および臨床像

1）高 LDL-C 血症

LDL 受容体など LDL 代謝に関わる対立遺伝子の一方に遺伝子変異を有するため、血中 LDL-C が著明に増加する。

2）冠動脈硬化症

FH ヘテロ接合体は無治療では心臓死が 60%前後と多く、男性では 30 歳以降に心筋梗塞がほぼ一定の割合で増加するが、女性では 50 歳以前では稀である。

3）腱・皮膚黄色腫

重要な身体所見は腱黄色腫、皮膚黄色腫である。黄色腫は、手・肘・膝関節の伸側、手首、臀部など機械的刺激が加わる部位に多く発生する。腱黄色腫はアキレス腱肥厚として現れることが多く、視診・触診が重要である。軟線撮影によりアキレス腱の最大径を測定し、9mm

以上で肥厚ありと診断することもできる。ただ、遺伝子検査で FH と確定診断されても20-30%の患者では黄色腫を認めないことから(2)、黄色腫がなくても FH を否定してはならない。なお、眼瞼黄色腫は FH に特徴的ではなく診断的価値は低い。

4)角膜輪

角膜輪も FH によくみられる所見であるが、その出現頻度は 3 割程度である。

Ⅰ-2. 原因遺伝子

LDL 受容体のほか、アポ蛋白 B(アポ B)、Proprotein Convertase Subtilisin/Kexin type 9 (PCSK9) の遺伝子変異で発症する。臨床診断された FH ヘテロ接合体の 60-80%に原因遺伝子の変異が確認される。

Ⅱ. FH ヘテロ接合体の診断

Ⅱ-1. LDL-C のカットオフ値

FH439 名、非 FH958 名、計 1397 名の未治療時データを解析し、LDL-C180mg/dL 以上、アキレス腱肥厚または皮膚黄色腫、2 親等内の血族に FH または若年性冠動脈疾患の存在を大項目として解析を行ったところ、感度 94.3%、特異度 99.1%であり、190mg/dL 以上の場合、感度 92.1%、特異度 99.1%であった。従って、特異度に変化がなく感度が高い LDL-C のカットオフとして 180mg/dL を採用した。診断基準を表 1 に、診断に至るフローチャートを図 1 に示す。なお、今回の解析で LDL-C が 250mg/dL 以上で非 FH 症例は 5%であり、LDL-C が 250mg/dL 以上であれば、それのみで FH を強く疑う。

Ⅱ-2. アキレス腱軟線撮影

アキレス腱肥厚の評価は軟線撮影で行う。下腿骨と足底が 90 度となるようポジショニングし、側面から腓骨外果中心に入射する。撮影距離は 120cm とし、撮影条件は 50kV、5.0mA とする。超音波を用いた評価も可能であるが、まだ標準化されていない。

Ⅱ-3. 鑑別診断

FH との鑑別を要する疾患は、続発性高脂血症をきたす疾患 (糖尿病、甲状腺機能低下症、ネフローゼ症候群など) と、類似疾患である家族性複合型高脂血症 (FCHL) である。FCHL は腱黄色腫を合併しないこと、small dense LDL の存在、家系内に他のタイプの脂質異常症 (IIa 型、IIb 型、IV 型) が存在すること、小児では LDL-C 値が FH ほど上昇しないことなどから鑑別しうる。

Ⅱ-4. FH ヘテロ接合体のリスク因子と LDL-C の管理目標値

FH 117 名の検討により、糖尿病と低 HDL-C 血症が (3)、別集団の解析により喫煙、冠動脈疾患の家族歴、アキレス腱肥厚、LDL-C 高値、低 HDL-C 血症、高トリグリセライド血症、糖尿病、高血圧が冠動脈疾患のリスクを上昇させることが示された。また、別のコホートで低 HDL-C 血症 (4)、糖尿病、耐糖能異常 (5)、内臓脂肪(6)の重要性も指摘されている。さらに原発性高脂血症研究班において冠動脈疾患を持つ FH において高トリグリセライド血症及び低 HDL-C 血症の合併が多かったことが報告されている(7)。また、Lipoprotein (a)が 50mg/dL 以上の FH で有意に心血管イベントが増加することも示されている。これらの研究成果をもとに、FH ヘテロ接合体の動脈硬化発症、進展を決定しうるリスク因子として、通常の冠動脈疾患のリスク因子に加えて、男性 30 歳以上、女性で 45 歳以上または閉経後、未

治療時の LDL-C260mg/dL 以上、15mm 以上のアキレス腱肥厚、Lipoprotein(a)50mg/dl 以上、メタボリックシンドロームを考慮すべきである。

脂質管理基準に関して FH はきわめて冠動脈疾患のリスクが高い疾患であることから 2 次予防に相当すると考え、LDL-C の管理目標値は、100mg/dL 未満とすることが望ましい。しかし、FH の診療において LDL-C が 100mg/dL 未満という管理目標を達成することは困難なケースが多いことから、LDL-C が管理目標値に到達しない場合、治療前値の 50%未満を目指すことも可とする。ただ、目標に到達していても、イベントが起こらないことを保障するものではない。なお、FH の治療を行う上においては日本動脈硬化学会から出されているリスクチャートによるリスク評価は適応できない。この治療目標は 30 歳以上の FH に適応し、その治療は原則として専門医の指導の元で行うことが望ましい。15 歳以上 30 歳未満の FH に対する治療は必ず専門医の指導のもとに行う。

Ⅲ. FH ヘテロ接合体の治療法

Ⅲ-1 非薬物療法

FH においても食事療法・運動療法は実践すべきであり、その詳細は別項(第 16 章)に詳述する。しかしながら、動脈硬化性疾患のリスクが高いため、運動療法を始める前に動脈硬化性疾患のスクリーニングが必要である。労作性狭心症の有無の問診、運動負荷心電図、心エコー検査等を行って動脈硬化性疾患の評価を行い、虚血性心疾患の存在が疑われるときには運動療法の前に虚血性心疾患の治療を優先する。また、禁煙、肥満対策も重要である。

Ⅲ-2. 薬物療法

スタチンが第一選択薬となる。FH ヘテロ接合体患者 329 名を対象に我が国で行われた後ろ向き解析でも、スタチンの使用が冠動脈疾患の発症を有意に遅延させることが示されている(8)。LDL-C の低下効率から考えると、第二世代スタチンがファーストラインの治療薬となる。スタチン単独投与で十分な効果が得られない場合、他の脂質低下薬を併用する。併用薬としては、エゼチミブや胆汁酸吸着レジン (コレスチラミンやコレスチミド)、プロブコール、フィブラート系薬剤、ニコチン酸製剤などを用いる。但し、これらの併用療法が、スタチンによる単独治療に比べて FH 患者の心血管イベントをより有効に抑制するエビデンスはない。また、プロブコールの使用が FH ヘテロ接合体における冠動脈疾患の再発を遅らせることが、後ろ向きの検討から示唆されている(9)。

Ⅲ-3. LDL アフェレシス治療の適応

FH ヘテロ接合体に対しては、総コレステロール値が食事療法下の定常状態において 400 mg/dL を超え、250 mg/dL 以下に下がらず、黄色腫を伴い冠動脈病変が明らかな場合、LDL アフェレシス治療を考慮すべきである。適応と判断した場合は必ず専門医にコンサルトする。

Ⅳ. 動脈硬化性疾患のスクリーニング、フォローアップ

FH ヘテロ接合体は冠動脈疾患を含む全身性の動脈硬化性疾患を早期に発症するため、それらの合併の有無をスクリーニングし、早期に治療する必要があり、1〜2 年毎に冠動脈疾患の有無について検査を行うことが推奨される。冠動脈疾患の診断には問診とともに、心電図、運動負荷試験 (マスター2 段階試験、エルゴメーター、トレッドミル等)、運動・薬物負荷心筋シンチグラム、心臓超音波検査等を施行する。虚血が疑われたときには MD-CT、冠動

脈血管造影を施行するが、FHにおいては冠動脈石灰化のため、MD-CTでの評価が困難な場合があり、注意が必要である。

頸動脈の粥状動脈硬化の評価には、診察での血管雑音の聴取とともに、頸動脈エコーを行い、狭窄が疑われる場合はMRA、CT angiography、血管造影等も行う。FHヘテロ接合体の高齢者では大動脈瘤なども合併することが多く、胸腹部大動脈瘤の評価を行うことも推奨される。動脈硬化症の診断の詳細に関しては第3章を参照のこと。

V．小児FH

V-1.　小児のFHヘテロ接合体の診断

FHヘテロ接合体に最初に現れる所見は高LDL-C血症であり、小児期にはアキレス腱黄色腫や角膜輪などの高LDL-C血症に伴う身体症状が現れない例が多い。そのため、小児FHは、主に高LDL-C血症と家族歴をもとに診断される。小児のFHを診断するには、高LDL-C血症を有する親がいれば、親のFHの診断を確定する。小児FHヘテロ接合体の診断基準を表2に示す。健常児の95%のLDL-C値が140 mg/dL以下であることから(10)、スクリーニングの判断基準値を140 mg/dLとする。

V-2.　小児FHヘテロ接合体の治療法

1. 栄養指導、生活習慣の改善

FHヘテロ接合体と診断すれば、できるだけ早期に患児および保護者に対して生活習慣の改善を指導する。喫煙の習慣を持つ患児に対して禁煙の指導をすることはもちろんのこと、生涯にわたって喫煙を避けることを指導し、受動喫煙のリスクを説明し、家族に対しても禁煙の指導を行う。

2. 薬物療法

FHヘテロ接合体患者に対し、何歳から治療を行なうべきかのエビデンスは我が国ではまだ確立していない。ヘテロ接合体においても冠動脈の粥状動脈硬化性変化は若年期より見られることから、若年期より適切なLDL-C管理を実施することが推奨される。米国小児学会の提言では“LDL-C 190mg/dL以上”または“LDL-C 160mg/dL以上で若年性粥状動脈硬化性疾患の家族歴か2つ以上の危険因子を有する”場合には、小児であっても脂質低下治療を開始すべきとし、生活習慣改善の効果が十分でない場合、8～10歳以上の男子または初経を迎えた女子には薬物治療も考慮するとしている(11)。なお、腱黄色腫や大動脈弁狭窄症の合併、粥状動脈硬化の著しい家族歴があるなどリスクが極めて高いと考えられる症例では、FHホモ接合体の鑑別診断を行うとともに、より若年期からのスタチンを中心とした薬物治療開始を考慮する。薬物治療は、成長・発育等への安全性の観点から、これまで消化管から吸収されない胆汁酸吸着レジン製剤が主に用いられてきており、第一選択薬とされる。小児FHに対する薬物療法は、専門医の指導のもとに行うべきである。

VI.　女性のFHヘテロ接合体

妊娠中における胆汁酸吸着レジン以外の薬物療法は胎児奇形などの発症リスクが懸念されるため慎重にすべきである。National Institute for Health and Clinical Excellenceによれば、薬物治療中に妊娠が判明した場合には胆汁酸吸着レジン以外の脂質低下薬はただちに中止するべきであり、薬物治療中で妊娠の可能性のある場合には、3ヶ月間の薬物投与