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Severe Hypercholesterolemia in a Double Heterozygote for Lipoprotein Lipase Deficiency (LPL_{Arita}) and Apolipoprotein ϵ_4 : A Report of a Family with LPL_{Arita}

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Abstract. Although heterozygous lipoprotein lipase (LPL) deficiency is not rare, only part of the phenotypes may have been reported in Japan. Here we describe a Japanese family with LPL_{Arita}, the most common mutation linked to familial LPL deficiency in Japan, and show for the first time a heterozygote for the mutation who had marked hypercholesterolemia due to increased low-density lipoprotein (LDL) cholesterol. The proband's mother, one of the heterozygotes for LPL_{Arita} in the family, had both severe hypercholesterolemia (total cholesterol 306 mg/dl) with an especially increase in LDL-cholesterol and mild hypertriglyceridemia (180 mg/dl). She had normal LDL receptor activity and did not show clear evidence of possible causes of secondary hyperlipidemia. In addition to being heterozygous for LPL deficiency, she was also heterozygous for apo ϵ_4 . Because the ϵ_4 allele is known to be associated with higher LDL-cholesterol, heterozygous apo ϵ_4 may be one of causes of her LDL-cholesterol elevation. The other three heterozygotes for LPL_{Arita} were moderate drinkers, and all of them had both remarkable hypertriglyceridemia and mild hypercholesterolemia due to increased very-low-density lipoproteins (VLDL). The results suggest that heterozygotes for LPL_{Arita} can exhibit various phenotypes of hyperlipidemia, that is, hypertriglyceridemia and/or hypercholesterolemia due to not only increased VLDL but also increased LDL. The phenotypes appear to depend on some other genetic and environmental factors.

Key words: Lipoprotein lipase deficiency, LPL_{Arita}, Hypercholesterolemia, Low-density lipoprotein (LDL), Apolipoprotein (apo) E₄

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Lipoprotein lipase (LPL) is an important enzyme that hydrolyzes triglycerides transported by chylomicrons and very-low-density lipoproteins (VLDL) [1–3]. Defective LPL activity therefore leads to massive accumulation of chylomicrons in plasma and a corresponding increase of the triglyceride concentration. A congenital defect in the enzyme, familial LPL

deficiency, is a rare autosomal recessive disorder that occurs in about one in every million persons worldwide. However, this disorder is known to occur frequently in Quebec [4] and occasionally in West Japan [5], and accordingly heterozygous LPL deficiency are expected to be relatively common in those areas.

Because obligate heterozygotes for LPL deficiency have about 50% less LPL activity than normal subjects [5, 6], they seem to have increased VLDL and decreased low-density lipoproteins (LDL). In reality, however, their plasma triglycerides vary from normal to moderately high [5, 6], and they often have

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increased plasma cholesterol [7]. In addition, Julien [4] has found that French-Canadian heterozygotes sometimes have increased LDL and intermediate-density lipoproteins. Thus, heterozygotes for LPL deficiency could exhibit various phenotypes, but no Japanese heterozygote who has remarkable increased LDL has been reported. Here we describe a Japanese family with the LPL_{Arita} mutation (deletion of the G at base 916 in exon 5) and show for the first time a heterozygote for the mutation who had marked hypercholesterolemia due to increased LDL-cholesterol.

Subjects and Methods

The proband, a 25-year-old Japanese man, was admitted to the Kanazawa Red Cross Hospital in February 1999 because of acute pancreatitis secondary to severe type V hyperlipidemia. His fasting serum triglyceride concentration was 3,549 mg/dl and his total cholesterol concentration was 305 mg/dl. Hyperlipidemia had been noted during infancy because of an episode of lower intestinal bleeding. He was diagnosed as having LPL deficiency because plasma LPL mass was not detectable by an enzyme immunoassay with the Markit-F LPL kit (Dainippon Pharmaceuticals, Osaka, Japan) when measured 10 minutes after a bolus injection of heparin (30 units/kg of body weight). We therefore examined his LPL gene for LPL_{Arita}, the most common mutation linked to LPL deficiency in Japan [5, 8] and a cause of complete deficiency of LPL synthesis [9]. For detection of LPL_{Arita}, exon 5 of the LPL gene was amplified by the polymerase chain reaction (PCR) and the amplified fragment was digested with *Alu* I [9]. The LPL_{Arita} allele was characterized by the loss of an *Alu* I site in exon 5. As we had expected, he was confirmed to be homozygous for LPL_{Arita}.

Two months later we met five of his relatives and obtained their unanimous consent to participate in our investigation after they were provided with detailed oral and written information. They were asked about their medical histories and their habits, and underwent physical examinations. Venous blood samples were collected in the morning after an overnight fast.

Serum triglycerides, cholesterol, apolipoproteins,

apolipoprotein (apo) E polymorphism, lipoprotein (a), free fatty acid (FFA), fasting plasma glucose, fasting serum insulin and hemoglobin A_{1c} were investigated in all subjects. Lipoprotein fractions were prepared by ultracentrifugation, and triglyceride and cholesterol concentrations were determined by enzymatic methods. Apolipoproteins and lipoprotein (a) were measured by turbidimetric immunoassay. Apo E phenotypes were determined by isoelectric focusing and apo E genotypes were done by *Hha* I digestion following PCR [10]. Serum FFA was assayed by an enzymatic method based on the activity of acyl-coenzyme A synthetase. LDL receptor activity in the proband's mother was further investigated by the flow cytometric assay [11].

Results

The family pedigree is shown in Fig. 1. Consanguinity was not detected in the proband's parents. There were two persons with coronary heart disease (CHD). One of the proband's uncles (II-4) died of acute myocardial infarction, and another uncle (II-5) underwent coronary artery bypass grafting when he was 56 years old. None of the other family members had developed heart disease and all of their electrocardiograms were normal.

The clinical characteristics of the proband and his five relatives are shown in Table 1. All of his relatives but II-2 were confirmed to be heterozygous for LPL_{Arita} by *Alu* I digestion of their PCR-amplified DNA from the LPL gene.

One of the proband's uncles (II-1) had been treated for hyperlipidemia. His severe hypertriglyceridemia had been noted due to acute pancreatitis when he was 44 years old. Since then, his serum triglyceride concentration had fluctuated between 300 mg/dl to 2,000 mg/dl in spite of administration of hypolipidemic agents. His serum apo B, apo C-II, apo C-III and apo E concentrations were all increased.

Our investigation revealed for the first time that the proband's mother (II-8) had hyperlipidemia. Her menopause had occurred several years before. Her serum triglyceride and total cholesterol concentrations changed from 152 mg/dl and 265 mg/dl to 180 mg/dl and 306 mg/dl, respectively, on different fasting days. Though the increase in triglycerides was only slight, LDL, especially LDL-cholesterol,

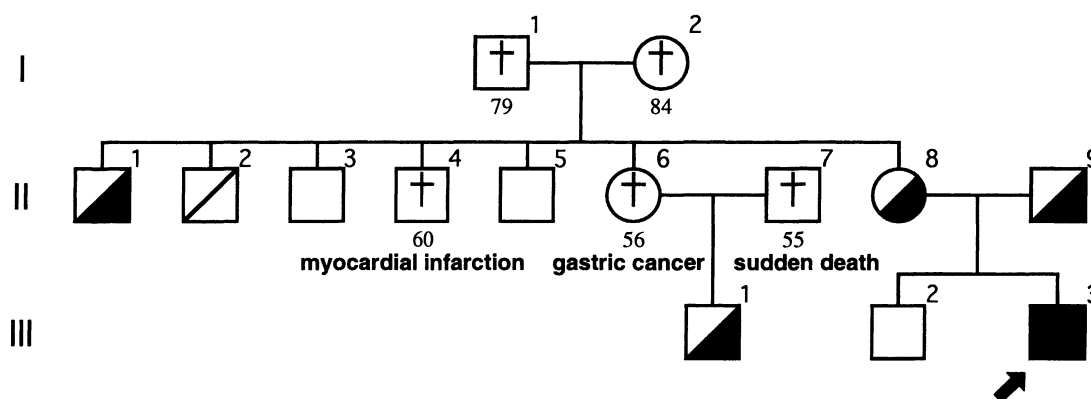


Fig. 1. Pedigree of the family. The proband is indicated by an arrow. ■, a homozygote for LPL_{ARITA}; ▨, heterozygotes for LPL_{ARITA}; □, normal; □, not studied; ⊕, deceased subjects. Numbers under the symbols indicate their ages that they died at. The names of diseases indicate their causes of death.

were substantial. Her serum apo B, apo C-II, apo C-III, apo E, FFA, and lipoprotein (a) concentrations were also increased. Apo E phenotype was E_{4/3} and apo E genotype was $\epsilon_{4/3}$. Her LDL receptor activity was 92% (normal range, $\geq 80\%$). She did not have hypothyroidism (TSH, 3.4 μ U/ml; free T₃, 3.1 pg/ml; free T₄, 1.3 ng/dl), nor did she show clear evidence of other possible causes of secondary hyperlipidemia.

The proband's father (II-9) was a moderate drinker with poorly controlled diabetes mellitus and hypertension. His fasting serum triglyceride concentration was 377 mg/dl and his total cholesterol concentration was 288 mg/dl. His hyperlipidemia was mainly the result of an increase of VLDL. His serum apolipoproteins and FFA concentration were also increased.

The proband's cousin (III-1) was also a moderate drinker. He had hypertriglyceridemia, markedly increased VLDL-triglyceride, and moderately increased apo B, apo C-II, apo C-III and apo E concentrations.

Discussion

This study has newly elucidated that heterozygotes for LPL_{ARITA} can have increased LDL. It has been reported that LDL modified by LPL is more actively taken up by the LDL receptor on macrophages than is unmodified LDL, leading to increased cell cholesterol content *in vitro* [12]. In addition, other data [13] suggest that LPL increases LDL uptake by

macrophages via a pathway not involving the LDL receptor. Furthermore, Williams *et al.* [14] have shown that LPL enhances reuptake of apo B in hepatoma cells. In view of these evidence, decreased LPL activity is likely to induce plasma LDL elevation. In fact, it has been reported that heterozygotes for LPL deficiency sometimes have increased LDL [4, 7]. However, another study [15] suggests that heterozygotes for LPL deficiency have only elevated plasma triglycerides, and Sniderman *et al.* [16] have concluded that they do not have increased LDL. Therefore, whether heterozygous LPL deficiency alone is associated with increased LDL remains controversial.

Among the heterozygotes for LPL_{ARITA} in the proband's family, the proband's mother had hypercholesterolemia due to increased LDL-cholesterol. Since her LDL receptor activity was normal, she was probably not a heterozygote for familial hypercholesterolemia. She was a postmenopausal woman, and the menopause may be one of causes of her LDL-cholesterol elevation because menopause is known to lead to a rise in the LDL-cholesterol level [17, 18]. In addition to being heterozygous for LPL deficiency, she was also heterozygous for apo ϵ_4 . The major role of apo E in lipoprotein metabolism is to serve as a ligand for the removal of lipoproteins from circulation via a receptor-mediated process [19]. The classical LDL receptor and the LDL receptor-related protein are involved with apo E-mediated interaction. While the ϵ_4 allele is known to be associated with higher total cholesterol, LDL-

Table 1. Clinical Characteristics of the Family Members

Subject number		II-1	II-2	II-8	II-9	III-1	III-3
Age	years	71	69	54	55	37	25
Sex		M	M	F	M	M	M
Mutation		Arita/W	W/W	Arita/W	Arita/W	Arita/W	Arita/Arita
Total triglycerides	mg/dl	347	203	152	377	437	3549
VLDL triglyceride	mg/dl	236.4	126.3	83.2	291.1	353.0	—
LDL triglyceride	mg/dl	84.7	60.2	52.5	59.3	59.2	—
HDL triglyceride	mg/dl	25.4	16.7	15.9	26.5	25.0	—
Total cholesterol	mg/dl	252	254	265	288	228	305
VLDL cholesterol	mg/dl	76.2	49.1	45.0	88.3	73.3	—
LDL cholesterol	mg/dl	138.8	164.7	175.5	147.4	113.8	94
HDL cholesterol	mg/dl	36.8	40.3	44.6	51.8	40.9	11
Lipoprotein (a)	mg/dl	35.9	25.6	63.6	6.8	33.8	1.7
Free fatty acid	mEq/l	0.72	0.78	1.08	1.08	0.52	0.95
Apolipoprotein A-I	mg/dl	130	129	128	188	139	73
Apolipoprotein B	mg/dl	161	154	166	153	141	42
Apolipoprotein C-II	mg/dl	10.5	6.3	6.7	13.2	8.9	8.7
Apolipoprotein C-III	mg/dl	15.5	10.5	10.4	29.3	21.8	18.0
Apolipoprotein E	mg/dl	8.2	6.5	4.7	8.7	8.9	11.4
phenotype		E4/3	E4/3	E4/3	E3/3	E3/3	E3/3
genotype		ϵ 4/3	ϵ 4/3	ϵ 4/3	ϵ 3/3	ϵ 3/3	ϵ 3/3
Fasting glucose	mg/dl	103	98	99	179	92	65
Fasting insulin	μ U/ml	8.3	12.2	8.9	4.1	7.2	2.6
Hemoglobin A _{1c}	%	5.1	5.4	5.2	8.0	4.8	—
Body mass index	kg/m ²	24.8	25.6	20.2	21.1	22.9	19.3
Blood pressure	mmHg	138/98	162/96	124/78	184/92	108/70	110/60
Clinical manifestations							
Xanthoma		(—)	xanthelasma	(—)	(—)	(—)	(—)
Pancreatitis		(+)	(—)	(—)	(—)	(—)	(+)
Habits							
Alcohol intake	kcal/day	160	(—)	(—)	560	480	(—)
Smoking		(—)	(+)	(—)	(—)	(+)	(—)
Drugs		bezafibrate	(—)	(—)	(—)	(—)	(—)
		400 mg/day					

W: wild-type allele, Arita: LPL_{Arita} allele.

cholesterol, and apo B than the ϵ_3 allele, the ϵ_2 allele has the opposite effect for these parameters [20, 21]. Therefore, heterozygous apo ϵ_4 seems to be another cause of the mother's LDL-cholesterol elevation. Further studies are needed to clarify whether heterozygotes for both LPL_{Arita} and apo ϵ_4 tend to have increased LDL-cholesterol.

Although heterozygous LPL deficiency is considered to lead to some of the cases of familial combined hyperlipidemia (FCHL) [7, 22, 23], Japanese patients with heterozygous LPL deficiency and FCHL have not been reported. One of the reasons

is that there have been no report of Japanese heterozygotes for LPL deficiency with increased LDL-cholesterol. LDL levels can be influenced by various genetic and environmental factors, including menopause and apo E polymorphism [18, 21]. The present study suggests that heterozygotes for LPL_{Arita} can have increased LDL at least under some conditions, and that heterozygous LPL deficiency might also lie behind FCHL in Japan.

All heterozygotes except the proband's mother had remarkable hypertriglyceridemia and also had hypercholesterolemia due to increased VLDL-cholesterol.

Since all of them were moderate drinkers and the proband's father had diabetes mellitus, and it is known that drinking and hyperinsulinemia have deleterious effects on plasma triglyceride levels in heterozygous LPL deficiency [5, 24], their increase of VLDL seems to be partly due to alcohol-induced overproduction of VLDL or insulin resistance-induced decrease of LPL activity.

Of additional interest is the fact that two persons in the family had CHD. Henderson *et al.* [25] recently reported that LPL activity is decreased in patients with CHD, and Nordestgaard *et al.* [26] have shown that an LPL mutation is more frequent among patients with CHD than among individuals in the general population. However, it is still unknown

whether heterozygous LPL deficiency predisposes carriers to CHD. Regrettably, we could not examine the two persons with CHD because one was no longer alive and the other was living abroad. This is another reason why we plan to carefully follow up the others in the family.

In summary, we have shown a Japanese family with familial LPL deficiency (LPL_{ARITA}), hyperlipidemia, and CHD. Heterozygotes for LPL_{ARITA} can exhibit various phenotypes of hyperlipidemia, that is, hypertriglyceridemia and/or hypercholesterolemia due to not only increased VLDL but also increased LDL. The phenotypes appear to depend on some other genetic and environmental factors.

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