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Letter to the Editor

Detection of a new compound heterozygote (del G^{916}/G1401A) for lipoprotein lipase deficiency and a comparative haplotype analysis of the mutant lipoprotein lipase gene from Japanese patients

Minoru Okubo [a,*], Shigeaki Inoue [b], Asako Horinishi [a], Takeshi Ogihara [b,1], Ken-ichiro Kaneko [b], Takanari Gotoda [c], Nobuhiro Yamada [c], Toshio Murase [a]

[a] *Department of Endocrinology and Metabolism, Toranomon Hospital, and Okinaka Memorial Institute for Medical Research, 2-2-2 Toranomon, Minato-ku, Tokyo 105, Japan*

[b] *Department of Pediatrics, Urayasu Hospital, Juntendo University, Chiba 279, Japan*

[c] *The Third Department of Internal Medicine, Faculty of Medicine, University of Tokyo, Tokyo 113, Japan*

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Dear Editors

A patient with early-onset chylomicronemia from a non-consanguineous family to be a compound heterozygote for two different mutations in the lipoprotein lipase (LPL) gene has been demonstrated. Reports on compound heterozygotes for LPL deficiency have been limited.

The patient, a 2-month-old Japanese boy, was admitted to Urayasu Hospital because of hyperlipidemia. He showed eruptive exanthomas on his face and buttocks, but had no signs of pancreatitis or hepatosplenomegaly. The patient's plasma triglyceride (TG) and total cholesterol (TC) concentrations were 14 183 and 763 mg/dl, respectively (Table 1). The apolipoprotein (apo) CII concentration was a predictable value for plasma TG levels from a previous study [1]. LPL activity, assayed as described elsewhere [2], and mass, determined with a sandwich enzyme-linked immunosorbent assay (ELISA) kit (Dai-nippon Pharmaceutical, Japan) [3], in the patient's postheparin plasma were virtually undetectable. The father's plasma lipid levels were within the normal range, whereas the mother exhibited moderate hypertriglyceridemia shortly after the delivery. The LPL activities of the parents were nearly normal, but their LPL masses were approximately half the normal value. Informed consent for molecular analysis was obtained from the parents.

Nucleotide sequence analysis of the patient's LPL gene showed two separate point mutations: a single guanosine deletion at nucleotide 916 (del G^{916}) in exon 5, leading to premature termination at codon 224 by a frameshift; and a G-to-A transition at nucleotide 1401 (G1401A) in exon 8, resulting in a substitution of tryptophan (TGG) at codon 382 by a stop codon (TGA). Southern blot hybridization was performed first, as described previously [4], but showed no major rearrangements (data not shown). The polymerase chain reaction (PCR) was then carried out as previously described [4]. PCR products were directly inserted into a plasmid vector pCRII (Invitrogen, USA), and the nucleotide sequence of subcloned plasmids were determined in both directions by dideoxy termination with an AmpliTaq cycle sequencing kit (Perkin-Elmer, USA)

* Corresponding author. Tel.: +81-3-3588-1111; fax: +81-3-3582-7068.

E-mail address: QFG00550@nifty.ne.jp (M. Okubo)

[1] Present address: Department of Medicine, Urayasu Hospital, Juntendo University, Chiba 279, Japan.

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using a DNA genetic analyzer PRISM 310 (Perkin-Elmer, USA). Analysis of subclones comprising exon 5 in the patient's LPL gene indicated that half of those examined had the del G^{916}, while the other half had a normal sequence (Fig. 1). Nucleotide analysis of subclones inserted in the patient's exon 8 showed that half of the subclones had a G1401A transition, while the other half had a normal sequence (Fig. 1). No mutations were found in other exons or exon-intron junctions. In order to confirm these mutations, analysis by restriction fragment length polymorphism (RFLP) was performed as described previously [4,5]. Mutational study of the patient's family showed that the del G^{916} was inherited from his father, and G1401A from his mother (data not shown). These results established that the patient was a compound heterozygote for two different LPL gene mutations. Combination of these two mutations in a single patient had never been re-

Table 1
Biochemical data in the lipoprotein lipase (LPL)-deficient kindred

	Father	Patient	Mother	Normal[a]
Age	24 years	2 months	24 years	
Triglyceride (mg/dl)	144	14 183	465	
Total cholesterol (mg/dl)	216	763	226	
HDL-cholesterol (mg/dl)	42	61	34	
Apo CII (mg/dl)	3.6	21.0	11.3	3.7 ± 1.3
LPL activity (μmol FFA/ml per h)	4.3	<0.6	5.2	6.4 ± 2.1
LPL mass (ng/ml)	139	<20	131	223 ± 66
H-TGL activity (μmol FFA/ml per h)	4.3	9.9	3.6	8.8 ± 2.9
Apo E genotype	3/3	3/3	3/3	

[a] Normal values are expressed as mean ± S.D.

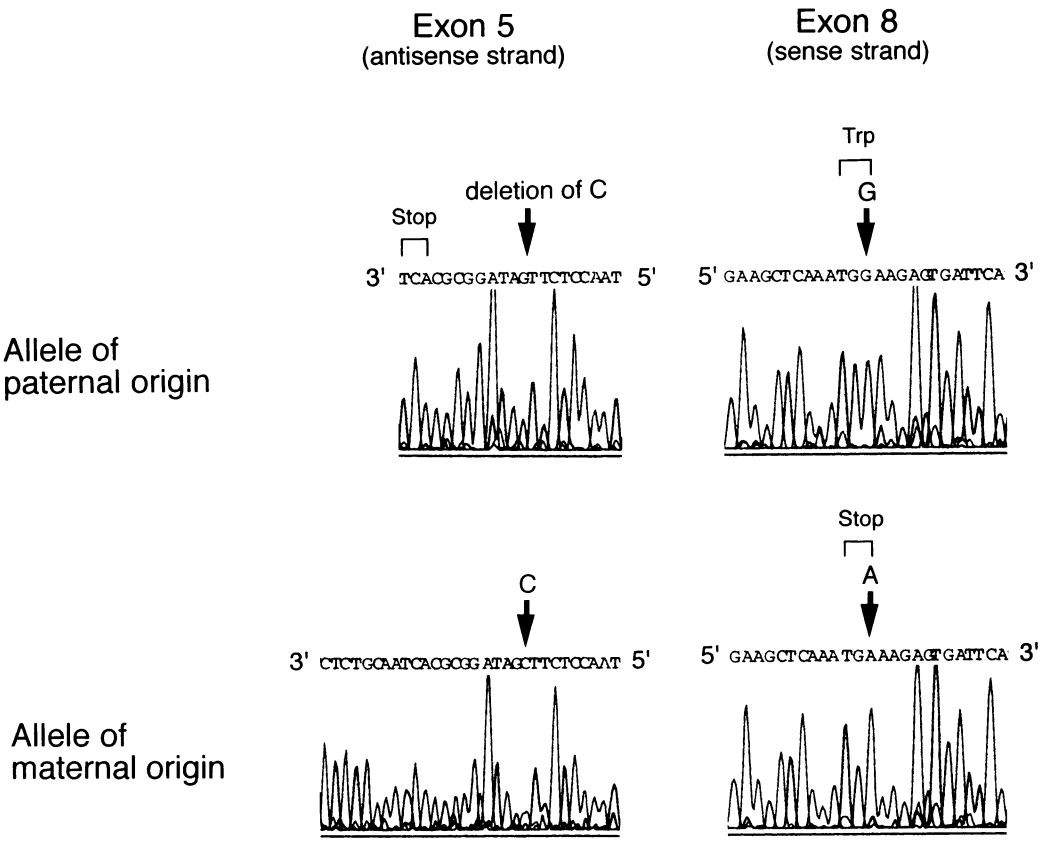

Fig. 1. Nucleotide sequence of the polymerase chain reaction (PCR)-amplified lipoprotein lipase (LPL) gene from the patient. The allele of paternal origin (upper panel) and the allele of maternal origin (lower panel) are identified by mutational analysis of the kindred. The sequence data in exon 5 in the antisense strand shows a deletion of G (C in antisense) in the allele of paternal origin, which results in a stop codon by a frameshift, and a normal sequence in the allele of maternal origin. The sequence data in exon 8 in the sense strand shows a normal sequence in the allele of paternal origin and a G-to-A transition in the allele of maternal origin, which causes the nonsense mutation. The patient was a compound heterozygote for the two mutations.

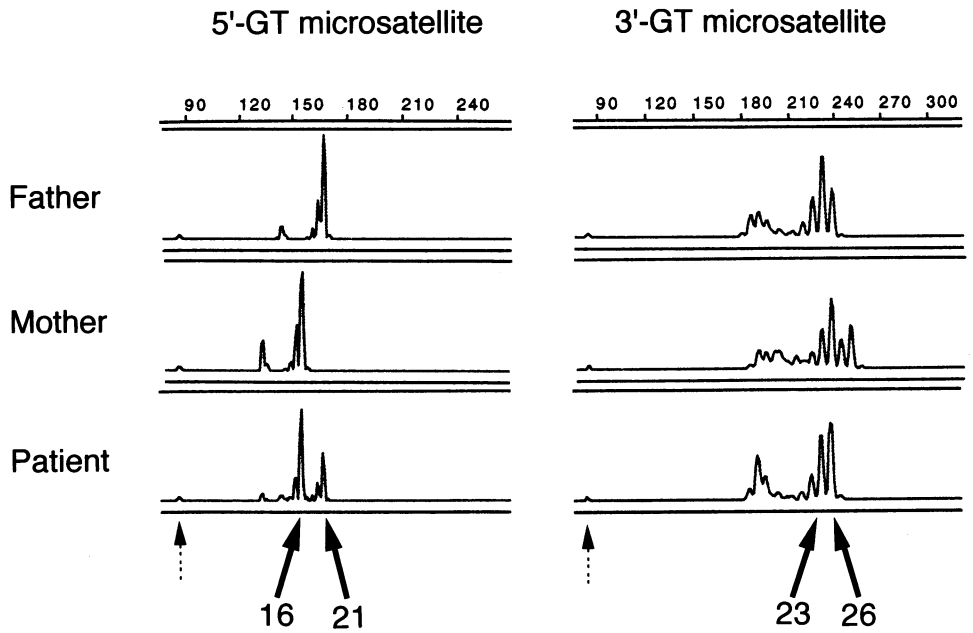

Fig. 2. Analysis of GT microsatellites flanking the lipoprotein lipase (LPL) gene analysis in the patient's kindred. The left panel shows the electrophoretic patterns from the LPL 5′-GT microsatellite, and the right panel shows the patterns from the 3′-GT microsatellite. The patient's GT repeats are indicated by arrows, and the exact numbers determined by sequence analysis are shown. The patient's 21 5′-GT repeats and 23 3′-GT repeats were inherited from the father, and the 16 5′-GT repeats and 26 3′-GT repeats from the mother. Arrows whose shafts are broken lines point to the peak of the size standard.

ported, although each of them had been reported independently previously [4–6].

The LPL gene mutations in this patient were thought to be null alleles, because virtually no LPL mass or activity was detected in the patient's postheparin plasma. This is consistent with previous results of in vitro expression studies. G1401A transition has been indicated to significantly reduce both LPL mass and activity [4,7]. Although the stop codon at codon 382 [W382X] in the patient was caused by the G1401A transition, the same G-to-A transition at nucleotide 1400, which also resulted in W382X, has been reported and shown to be a null allele as well [8]. Previous reports showed that G^{916} deletion caused a null allele [5,6]. These results were compatible with the findings that the patient's LPL mass and activity were undetectable.

The patient's LPL gene haplotype was investigated in order to compare it with those of apparently unrelated LPL-deficient patients in Japan with the same LPL mutation [4,5]. The del G^{916} mutation was previously reported in two families with LPL deficiency [5,6], and the family adds one more case of the del G^{916} mutation. The G1401A transition was also found in a homozygous patient with LPL deficiency [4]. These findings raised the question of whether the mutated alleles originated from a common ancestor or had occurred independently. RFLP haplotyping, constructed using *Pvu*II and *Hin*dIII RFLP by PCR and *Bam*HI RFLP by Southern blot hybridization as described previously [4,9], showed that the patient was *Hin*dIII (+/+), *Bam*HI (−/−), *Pvu*II (+/−). Analysis of the patient's family indicated that the del G^{916} allele had the haplotype A [*Hin*dIII (+), *Bam*HI (−), *Pvu*II (−)], while the G1401A allele had the haplotype C [*Hin*dIII (+), *Bam*HI (−), *Pvu*II (+)], according to a previous report [4]. These findings suggested that the patient's del G^{916} and G1401A allele had RFLP haplotypes identical to those of unrelated patients with the same mutation.

GT microsatellite haplotypes were further analyzed in the LPL gene. Polymorphic GT microsatellites have been reported to be more informative markers than RFLPs at the LPL locus [10,11]. The numbers of 5′- and 3′-GT microsatellite repeats in the patient's LPL gene alleles, determined by sequencing analysis, were 16 and 21 5′-GT and 23 and 26 3′-GT repeats. The size of the GT microsatellite alleles of the patient's parents and of families was compared with LPL deficiency previously reported [4,5], primarily based on the method of Wood et al. [10], which was modified by using fluorescence end-labeled primers, GS-500 as a size standard, and Performance Optimizing Polymer 4 on DNA genetic analyzer PRISM 310, according to the manufacturer's instructions. Analysis of microsatellites with the DNA genetic analyzer showed the relative sizes of the GT repeats, and comparison of the patient's and parents' microsatellites established that the del G^{916} allele had 21 repeats of the 5′-GT microsatellite and 23 repeats of the 3′-GT microsatellite, while the G1401A

Table 2
Distribution of 44 unrelated Japanese lipoprotein lipase (LPL) chromosome haplotypes and the LPL-deficient kindred's haplotypes

LPL 3′-GT	LPL 5′-GT						Total
	11	16	17	20	21	22	
21	0	1	0	0	0	0	1
23	0	3	0	0	1 (del G^{916})	0	4
26	1	23 (G1401A)	1	1	0	0	26
29	0	8	0	0	0	0	8
32	0	2	1	0	2	1	6
35	1	1	0	0	0	0	2
38	1	0	0	0	0	0	1
Total	3	38	2	1	3	1	48

allele had 16 repeats of the 5′-GT microsatellite and 26 repeats of the 3′-GT microsatellite (Fig. 2). These GT microsatellites were compared in the del G^{916} mutation in our patient and an LPL-deficient patient heterozygous for the del G^{916} mutation [5], and found that they were the same. The patient's G1401A allele had a GT microsatellite haplotype identical to that of a patient with the same mutation [4]. In addition, the GT microsatellite haplotypes were examined in 11 unrelated Japanese families. The distribution of 44 Japanese LPL chromosome haplotypes plus four haplotypes from the patient's kindred are shown in Table 2. These haplotype analyses disclosed two points: (1) the G1401A allele was on the commonest GT microsatellite haplotype among Japanese, whereas the del G^{916} allele was on a unique microsatellite haplotype; and (2) each patient's mutated allele had a haplotype identical to that of the patient with the same mutation, who is unrelated to the one described here. These findings suggest that the del G^{916} mutation had a common Japanese ancestor, and that the G1401A transition may also have originated from a common ancestor. However, the GT microsatellite haplotype on the del G^{916} mutation was not found among 44 chromosomes, indicating that the del G^{916} mutation is not very prevalent in the Japanese population, even though this mutation has occasionally been found in patients with LPL deficiency.

Just recently, a systemic survey of nucleotide-sequence variation in 71 individuals from three populations reported 88 variable sites in a 9.7-kb region of the LPL gene, and a total of 88 distinct haplotypes were identified [12]. Because more polymorphic markers and more independent chromosomes should be investigated to characterize all the possible haplotypes which occur in the Japanese population, further studies will be needed to define the identity of the haplotypes of the LPL-deficient patients mentioned above.

Heterozygous states of LPL mutations have attracted attention as one of the causes of postprandial hyperlipidemia [13]. Hyperlipidemia during pregnancy has been found to occur in women with heterozygous LPL mutations [8], and the patient's mother may be an example, because moderate hypertriglyceridemia was observed shortly after delivery. As the apo E isoform has been shown to influence expression of hyperlipidemia [8,14,15], the apo E genotype of the patient's family was determined by digestion of PCR-amplified DNA with *Hha*I according to Hixon and Vernier [16], and found the mother's genotype to be E3/E3, a wild-type isoform. This may explain why her hyperlipidemia was not severe.

In summary, a new case of familial LPL deficiency was described and a founder effect by performing haplotype analysis of the patient was investigated.

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