Abetalipoproteinemia Caused by Maternal Isodisomy of Chromosome 4q Containing an Intron 9 Splice Acceptor Mutation in the Microsomal Triglyceride Transfer Protein Gene

Xiao Ping Yang, Akihiro Inazu, Kunimasa Yagi, Kouji Kajinami, Junji Koizumi, Hiroshi Mabuchi

Abstract—Uniparental disomy (UPD), a rare inheritance of 2 copies of a single chromosome homolog or a region of a chromosome from one parent, can result in various autosomal recessive diseases. Abetalipoproteinemia (ABL) is a rare autosomal recessive deficiency of apoB-containing lipoproteins caused by a microsomal triglyceride transfer protein (MTP) deficiency. In this study, we describe a patient with ABL inherited as a homozygous intron 9 splice acceptor G(-1)-to-A mutation of the transfer protein gene. This mutation alters the splicing of the mRNA, resulting in a 36 amino acids, in-frame deletion of sequence encoded by exon 10. We analyzed chromosome 4, including MTP gene (4q22-24), using short tandem repeat markers. The proband has only his mother’s genes in chromosome 4q spanning a 150-centimorgan region; ie, segmental maternal isodisomy 4q21-35, probably due to mitotic recombination. Nonpaternity between the proband and his father was excluded using 6 polymorphic markers from different chromosomes (paternity probability, 0.999). Maternal isodisomy (maternal UPD 4q) was the basis for homozygosity of the MTP gene mutation in this patient. (Arterioscler Thromb Vasc Biol. 1999;19:1950-1955.)

Key Words: abetalipoproteinemia ■ microsomal triglyceride transfer protein ■ mitotic recombination ■ splicing mutation ■ uniparental disomy

Abetalipoproteinemia (ABL) is a rare autosomal recessive disorder of lipoprotein metabolism,1-3 characterized by extremely low levels of serum cholesterol and triglyceride, and the absence of apoB-containing lipoproteins. Clinical manifestations include malabsorption, neurological symptoms such as spinocerebellar dysfunction, retinopathy that causes impairment of night and color vision, and malformed erythrocytes (acanthocytosis). Microsomal triglyceride transfer protein (MTP) gene mutations have been shown to cause ABL4,5; among fewer than 100 patients with ABL, more than 13 distinct mutations of the gene have been reported so far, and genotype-to-phenotype relation has been shown recently.4-9 However, one-third of reported families were consanguineous.1 In addition, male ABL patients outnumber females about 6:4, suggesting some mechanism involving sex chromosomes or parent of origin differences may be operative in some cases.3

In contrast to classical Mendelian inheritance, uniparental disomy (UPD) is the inheritance of 2 copies of a region of chromosome from only one parent, without any contribution from the second parent. Inheritance of a pair of homologous chromosomes from one parent is uniparental heterodisomy. Inheritance of 2 copies of a single chromosomal homolog from one parent is defined as uniparental isodisomy.10,11 UPD may be associated with either an abnormal or a normal phenotype.12 UPD case reports may provide a clue to study of human genomic imprinting,12,13 an important mechanism of gene regulation.14 Isodisomy may result in a higher risk of autosomal recessive disorder than would heterodisomy.

We report here the first case of MTP deficiency caused by maternal UPD. The patient is homozygous for an intron 9 splicing mutation. He inherited 2 identical mutant alleles from his mother via maternal isodisomy of chromosome 4q. The MTP gene maps to 4q.4,8 Even though UPD has been documented for 17 human autosomes and the X and Y chromosomes, resulting in 18 recessive diseases, including LPL deficiency,12,15 only 1 case of isochromosome 4 (46, −4, −4, +i4q, +i4p) has been reported.16 In both cases of UPD4, phenotype did not suggest any abnormalities due to imprinting.

Methods

Subject
The proband, a 29-year-old Japanese male, was admitted for evaluation of low serum cholesterol concentration. His birth weight was...
normal (3200 g). Loss of night vision was suspected by the time that he was 3 years old. He had frequent diarrhea, fat malabsorption with malnutrition, short stature, and a history of anemia dating from childhood. His parents were unrelated. At the time of his birth, his mother was 26 years old and his father was 30. At the time he was examined by us, he was 152 cm (−3SD) tall and weighted 45 kg (−2SD). Neurologically, cerebellar and posterior spinal column dysfunctions were evident. Deep tendon reflexes were decreased, and vibratory sense and proprioseception were impaired; dysmetria, ataxia, and spastic gait were noted. A Chaddock sign was elicited. Ophthalmologic examination revealed decreased vision in dim light, visual field defects, and pigmented retinal degeneration. Scanning electron micrographs of peripheral blood showed acanthocytosis. Examination of an intestinal biopsy specimen disclosed lipid-laden enterocytes.

**Serum Chemistry Analyses**

Serum cholesterol and triglyceride concentrations were measured by enzymatic methods. HDL cholesterol (HDL-C) was determined by a heparin-calcium chloride precipitation method. Apolipoprotein levels were measured by immunoturbidimetry. Vitamin A and E levels were determined fluorometrically. Vitamin K levels were determined by high-performance liquid chromatography. Vitamin E level was determined fluorometrically.

**Mutation Analysis**

From the total RNA extracted from proband’s jejunal biopsy specimen, a full-length MTP cDNA was obtained through reverse transcriptase-polymerase chain reaction (SuperScript, BRL Gibco) using a pair of primers based on published cDNA sequence. The product was subcloned into a Bluescript vector. Wild type pRC/hMTP cDNA was kindly provided by Dr John R. Wetterau (Bristol-Myers Squibb, Department of Metabolic Diseases, Princeton, NJ), and used as the control cDNA sequence. Genomic DNA was extracted from peripheral blood leukocytes by a phenol-chloroform method and used as the template for polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis, a fragment of the junction of intron 9 and exon 10 was amplified by PCR using an intron 9 forward primer, 5’-AGAATTCCTTACACAGGT-3’, and a mismatch reverse primer of 5’-CCAATAAGACCTTCTTGAAGCTACT-3’. The 250 base pair fragment was digested with Hinf I, loaded on to a 10% acrylamide gel for electrophoresis, and visualized with ethidium bromide.

**Testing for UPD**

Short tandem repeat (STR) markers for chromosome 4 were obtained from the Cooperative Human Linkage Center (http://www.chlc.org/). STR fragments were amplified using genomic DNA as a template and pairs of primers, with one primer of each pair being labeled with fluorescein isothiocyanate. After denaturing at 95°C, the amplified fragments were loaded on a denaturing polyacrylamide gel and detected using Genescan software (PE Applied Biosystems).

Paternity testing was performed using 3 STR markers from 3 different chromosomes (chromosomes 5, 7, and 13; Geneprint Fluorescent STR System, Promega, Madison, WI), apo E polymorphism (chromosome 19), and 2 specific Y chromosome markers. The analysis was based on the published gene frequencies for general Japanese populations, using PT-TRIO software to calculate the probability of paternity between the proband and his father.

**Cytogenic Analysis**

Karyotypings, G-banding and high resolutional analyses, were performed on cultured blood lymphocytes from the proband.

**Biochemical Analyses**

Serum concentration of lipid and apolipoprotein are shown in Table 1. Levels of serum fat-soluble vitamins in the proband were vitamin A 22 IU/dL (normal range 65 to 275), vitamin E <2 µmol/L (<0.1 mg/dL) (normal range 17 to 33 mol/L; 0.75 to 1.4 mg/dL), vitamin K1 5.0 ng/dL (normal range 15 to 125), vitamin K2 5.0 ng/dL (normal range >10.0), and vitamin D 5.9 ng/dL (normal range 2.0 to 7.5). The proband had low levels of serum cholesterol and undetectable levels of serum triglyceride, apo B, and vitamin E.

**MTP Gene Analyses**

Compared with the wild-type sequence, the proband’s MTP cDNA has a deletion of 36 consecutive amino acids encoded by exon 10 (Figure 1). This deletion is expected to result in a complete deficiency of MTP activity. Direct sequencing of exon 10 and the junction of intron 9 and exon 10 showed a G-to-A transition at the splice acceptor site (−1) in the proband MTP gene (Figure 2). The proband had an additional G to T transversion in the intron

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**Figure 1.** Illustration of MTP cDNA sequence analysis. The upper panel represents the proband’s MTP cDNA sequence; the lower panel, the normal sequence. Compared with normal sequence, an exon 10 deletion is found in the proband’s MTP cDNA.
9 splice position (−7). The proband was homozygous for the intron 9 splice G(−1)-to-A mutation; his mother, with 1 normal base G and 1 mutant base A, was a heterozygote, whereas his father was homozygous for the normal base G. PCR-RFLP analysis was used to confirm this mutation using Hinf I digestion. The junction of intron 9 and exon 10 of MTP gene was amplified with a pair of primers. The mismatch primer created a Hinf I restriction site in the normal sequence, which is lost in the mutant sequence because of the G-to-A transition. Digestion of the PCR products with Hinf I resulted in either a 230 bp wild-type sized fragment or a 250 bp mutant-sized fragment. The proband produced a single 250 bp mutant-sized fragment. His mother, a sister, and his maternal grandfather produced 2 fragments, both the 230 bp wild-type and the 250 bp mutant fragment, indicating heterozygotes. A partial pedigree of this family for MTP deficiency is shown in Figure 3. His father was proven to be homozygous for the wild-type allele of the mutation site, despite a presumed obligate heterozygote. This result indicates that the proband inherited the recessive MTP mutation only from his mother.

Maternal Isodisomy of Chromosome 4q21-35

The MTP gene has been mapped to chromosome 4q22-24. The proband’s karyotype was normal by G-banding, and high-resolution analyses of 4 pairs of chromosome 4 revealed no obvious deletion. As the mutation from the proband’s mother was inherited from her father and the mutation was absent in the paternal branch of the proband’s family (Figure 3), we propose that maternal disomy for chromosome 4 is responsible for the homozygosity of the mutant MTP gene that results in ABL in this patient. UPD testing was carried out with STR markers on chromosome 4, as shown in Figure 4 and Table 2. The informative markers D4S1647, GAGT62A12, D4S2392, D4S2395, D4S2378, D4S2428, D4S2368, and D4S2417 show that the proband possesses only the mother’s alleles in the chromosome region 4q21-35. The father’s alleles are absent from the proband in this region. All the markers showed reduction to homozygosity over 150 centimorgans in the proband’s chromosome 4q. However, the proband inherited biparental alleles in 4 of polymorphic markers (vWFA, TPOX, THO1, and Apo E) from different chromosomes of 5, 7, 13, and 19. He is also a biparental heterozygote for the 4p and centromeric markers of D4S2633 and D4S2379 (Table 2). Results from STR markers from 4p16-q13 indicate that this region was inherited biparentally, and UPD was not via maternal heterodisomy.

![Figure 2. An intron 9 splice acceptor G(−1)-to-A mutation in the MTP gene. Direct sequencing was used to analyze the genomic DNA from the proband and his parents. The junction sequence of intron 9 and exon 10 of the MTP gene is shown. The exon 10 sequence and intron 9 sequence are shown in upper case and lower case letters, respectively. The bars indicate the splice acceptor bases G and A. The proband has a mutant base A (right). His mother has both mutant A and normal G (center). However, his father has only the normal base G (left).](http://atvb.ahajournals.org/)

![Figure 3. The pedigree of ABL caused by an intron 9 splice acceptor G(−1)-to-A mutation in the MTP gene. Arrow indicates the proband, a 29-year-old, homozygous for the mutation. His younger sister, mother, and maternal grandfather are identified as heterozygotes. His father and paternal grandparents are normal for the mutation.](http://atvb.ahajournals.org/)

![Figure 4. Illustration of maternal isodisomy of chromosome 4q. The STR fragments were amplified by polymerase chain reaction with fluorescent labeled primers. After denaturing, the products were loaded on 4% denaturing polyacrylamide gel, and detected by the Genescan method. M indicates mother; P, proband; and F, father. The numerals are designations of STR markers. Slender bars indicate the location of markers on the chromosome 4. The cross hatched region indicates the location of the MTP gene. These STR markers show that the proband inherited only a single homolog from his mother in chromosome 4q21-35. However, biparental inheritance is evident in the markers D4S2633 and D4S2379 in the proband (left panel).](http://atvb.ahajournals.org/)
Paternity testing, including analyses of 4 markers of autosomes and markers of YAP and DYS19 in chromosome Y, indicated a paternity probability of 0.999 between the proband and his father.

Discussion

A proband with a low level of serum cholesterol showed typical features of ABL: spinocerebellar symptoms, pigmentary retinal degeneration, acanthocytosis, malabsorption of fat, and deficiency of apoB-containing lipoproteins and vitamin E. Based on normal phenotype shown by his parents, he was diagnosed as recessive ABL rather than homozygous hypobetalipoproteinemia, which is an autosomal dominant disorder of apoB deficiency caused by apoB gene mutation.28 Analysis of cDNA isolated from the proband’s intestinal RNA showed that sequence encoded by exon 10 of the MTP gene was deleted. Genomic DNA sequence analysis revealed that an intron 9 splicing acceptor G(2)-to-A mutation was responsible for the exon 10 skipping in the cDNA. His mother was a heterozygote, though surprisingly, his father was found to carry no mutation. UPD testing showed that the proband is homozygous for MTP deficiency caused by maternal isodisomy for the region of chromosome 4q that codes for the mutant MTP gene.

Four splice donor site mutations have been reported in patients with homozygous MTP deficiency, so far. These patients were diagnosed in infancy. They took large doses vitamin E, and neurological findings were not remarkable.5,8,29,30 Our patient had a splice acceptor mutation, but was diagnosed in his late 20’s, and presented a prominent neurological syndrome. The serious neurological symptoms may have resulted from long-term vitamin E deficiency. Alternatively, the proband may have another recessive disease with deteriorating neurological symptoms due to the maternal isodisomy.

UPD can result from one of several mechanisms10,11,31–35: (1) Fertilization of a nullisomic by a disomic gamete, (2) chromosome duplication in a monosomic somatic cell after a postzygotic loss of one homologous chromosome (ie, monosomy rescue), (3) loss of a supernumerary chromosome in a cell with aneuploidy (ie, trisomy rescue), and (4) mitotic recombination in somatic cells that results in UPD for a portion of a chromosome (partial UPD). The mechanisms of monosomy or trisomy rescue result in complete UPD, whereas somatic recombination results in partial UPD. Because a parental contribution to chromosome 4, from 4p to the centromere, of the proband was indicated by 2 informative markers (Table 2), mitotic recombination at an early division between 4q13 and 4q21 appeared to be a plausible mechanism for this case, as shown in some cases of Beckwith-Wiedemann syndrome (paternal UPD 11p).34,35 Although some cases of Beckwith-Wiedemann syndrome are somatic mosaics, this was not evident in our proband. No wild-type MTP cDNA was obtained from jejunum RNA, and there were no additional DNA fragments seen in the STR marker analyses using genomic DNA from peripheral blood cells (Figure 4). Also, the severe phenotype of this case of ABL is inconsistent with mosaicism of chromosome 4q isodisomy.

UPD is calculated to occur in 2.8 of 10 000 conceptions.10 UPD as the cause of an abnormal phenotype should be considered, when a rare recessive disease is diagnosed.

### Table 1. The Levels of Serum Lipid and Apolipoproteins

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<th>Mother</th>
<th>Father</th>
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<td>Cholesterol (mg/dL)</td>
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<td>3.1 (118)</td>
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<td>Triglyceride (mg/dL)</td>
<td>0 (0)</td>
<td>0.6 (52)</td>
<td>3.7 (329)</td>
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<td>HDL-C (mg/dL)</td>
<td>0.7 (28)</td>
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<td>0.4–1.7 (32–150)</td>
<td>0.9–2.2 (34–86)</td>
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### Table 2. Genotype Analysis for Chromosome 4 STR Markers in the Proband and His Parents

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Number of repeat of STR is shown in alleles. Evidence for biparental inheritance is indicated by a long box. Evidence for UPD is indicated by a short box in the proband column.
without consanguinity. Many UPD cases are associated with specific syndromes or growth retardation, resulting from imprinting genes or loci. In cases of Prader-Willi syndrome (PWS), the reported frequency of maternal disomy is 25% and that of paternal deletion is 75%, which indicated that loss of paternal genetic material caused PWS. Previous reports of UPD expressing the recessive cystic fibrosis gene with short stature have indicated genomic imprinting in chromosome 7.31,36,37 The human chromosome 4 region has been reported to be homologous to a portion of mouse chromosome 3 that is not implicated in parent of origin differences.38 As only a report of UPD4, a woman with multiple early miscarriages had maternal isochromosome 4p and 4q.16 She was the tallest among her siblings but was otherwise normal. Our proband appeared normal except for manifestations of ABL, including small stature and low body weight. As compared with 3 Japanese male patients with ABL reported, our proband’s birth weight 3200 g was not different from 2780±630 g [SD], but height 152 cm and weight 45 kg at our proband’s age of 29 years were somewhat smaller than 164±13 cm and 56±14 kg at their mean age of 26±8 years, respectively. Thus, we believe that there are no obvious phenotypic effects of the UPD on our proband, but some effect on growth failure cannot be ruled out. In addition, we could not exclude the possibility that some region of chromosome 4 could be paternally inactivated, because no case of paternal UPD of chromosome 4 has been reported to date.

In conclusion, we determined that the proband in our study suffered from ABL that resulted from a homozygous mutation of the MTP gene. The proband was homozygous for the MTP mutant allele because of maternal UPD 4q21-35 region probably caused by mitotic recombination. This case study may provide a clue to explain phenotypic variability among ABL patients.

Acknowledgments

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