Original Article

Unique maternal deletion of 15q in a patient with some symptoms of Prader-Willi syndrome

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Abstract

Background: Human chromosome 15q11-q13 is a critical region for Prader-Willi syndrome (PWS) and Angelman syndrome (AS) and most of the genes are under the condition of imprinting mechanism. PWS results from the loss of expression of paternally expressed genes and AS of maternally expressed genes. In this study molecular studies about a patient with congenital anomalies and mental retardation are analyzed.

Methods: Highly polymorphic microsatellite markers were analyzed by PCR. These markers exist within 15q11-q13 and distal to 15q13.

Results: Only the maternal D15S986 locus within 15q11-q13 was deleted and other markers were biallelic.

Conclusions: The result of maternal small region deletion in this patient is different from the typical PWS with paternal chromosome deletion and it suggests that nearby the deleted region there exists a gene (genes) which is not imprinted but needs biallelic expression.

Key words deletion, microsatellite marker, Prader-Willi syndrome, uniparental disomy.

Materials and methods

Clinical summaries

The male patient, who is now an 18-year-old, was born after 40 weeks of gestation as the offspring of healthy parents without consanguinity. Although his birth weight was 2722 g without asphyxia, he showed minor anomalies and his sucking was poor. He underwent an operation for hypospadia at 4 months old. When he was introduced to Okayama University Hospital, Okayama, Japan, at 1 year and 7 months, he showed microcephaly, short neck, webbed neck, low set of ears, down slanting palpebral fissures, bilateral convergent
strabismus, bilateral II to V fingers skinny syndactyly, and cryptorchism. In his development he achieved the capability of rolling, but he was hypotonic and could not sit alone. He spoke no meaningful words. The chromosomal analysis using high resolution banding by the GTG banding method showed no deletion of 15q11-q13 and the fluorescent in situ hybridization (FISH) using a commercially available probe, LSI Prader-Willi/Angelman Region SNRPN (VYSIS; Woodcreek, USA), which is a more effective method, also revealed no deletion. He walked unaided at 3 years old, could run at 4 years old, could manage to follow easy directions and spoke meaningful words at 7 years old, and spoke two continuous word phrases at 13 years old. His hyperphagia has been marked since about 12 years old; at 13 years old his height was 147 cm (–0.85 SD) and his weight was 56.4 kg (+1.39 SD), and was 65 kg (+1.75 SD) at 14 years old. He had some clinical features for PWS (Fig. 1), so we tested for the possibility of mUPD of chromosome 15 using DNA microsatellite markers.

**Molecular studies**

Blood samples were obtained from the patient and the patient’s parents under informed consent of the parents. Genomic DNA was extracted by the standard method. Highly polymorphic microsatellite markers were analyzed by PCR. These markers are D15S11, D15S128, D15S122, D15S210, D15S986, D15S1234, GABRB3, D15S165 within 15q11-q13, and D15S126, D15S153, D15S211, D15S127 distal to 15q13.7 All PCR reactions were performed in a total volume of 20 µL using 5 pmol of each primer, 200 µmol each of dNTP, 1.25 mmol MgCl2, 50 mmol KCl, 10 mmol Tris-HCl (pH 8.3), 0.1 µL deionized formamide, 25 ng genomic DNA and 1 U AmpliTaq Gold (Perkin-Elmer, Foster City, CA, USA). The PCR cycling profile used was as follows: preheating at 95°C for 9 min, followed by 28 cycles of 94°C for 30 s, 58°C for 30 s and 72°C for 1 min and final extension at 72°C for 10 min. After amplification, the PCR product was mixed with an equal volume of formamide loading buffer consisting of 95% deionized formamide, 10 mmol EDTA, 0.1% xylene cyanol and 0.1% bromphenol blue, denatured at 95°C for 5 min, and chilled on ice. Each sample of 3 µL was loaded onto denaturing gels made of 7.7 M urea, 6% acrylamide, 1× TBE, 30% deionized formamide and the electrophoresis condition was 45 W constant watt. The gel was stained using a silver stain kit (DAIICHI, Tokyo, Japan).

The DNA methylation pattern was investigated for two loci. The two probes were provided through the American Type Culture Collection (ATCC); SNRPN (ATCC number: 95679) and PW71B (ATCC number: 99413). In both SNRPN and PW71B the cytosine residues from maternal allele are methylated. Genomic DNA was digested with XbaI + NotI (SNRPN) or with HindIII + HpaII (PW71B), run on a 0.8% agarose gel, transferred to a nylon membrane. The membrane was hybridized with 32P-DNA probes labeled by the random primer labeling method, Ready-To-Go DNA Labeling Beads (Amersham Pharmacia, Piscataway, NJ, USA). The wash condition was at room temperature in 2× SSC, and at 65°C in 0.1× SSC and 0.1% SDS, then the membrane was exposed to X-ray film at –70°C overnight.

**Results**

**Microsatellite marker analysis**

Microsatellite marker analysis is summarized in Table 1. It demonstrated biparental inheritance at three loci within 15q11-q13 (D15S11, D15S210, and D15S1234) and at three loci distal to 15q13 (D15S126, D15S153 and D15S211). Only one locus, D15S986, within 15q11.2-q12, derived from his mother, showed deletion. These electrophoreses of the biparental and maternal deleted inheritance loci in the patient are depicted in Figure 2. We excluded the possibility that one
Atypical PWS with small maternal deletion

Allele could not be amplified for point mutation within the primer sequence. D15S986 primers as follows: forward 5'-gcaggaatatgtccaggg-3' (Ori-F) and reverse 5'-catggctggtctttaggtg-3' (Ori-R). We used the primer Mix-F and the primer Mix-R which has base mixes (a, t, and c) substituted for g at the 3' end site of Ori-F and Ori-R. No PCR products could be amplified by using combinations of Ori-F and Mix-R, Mix-F and Ori-R, and Mix-F and Mix-R. Also in normal controls (25 males and 25 females) the combinations of the above primer sets had no amplified PCR products, so the possibility of polymorphism was denied. These findings show that the patient has maternal deletion at D15S986.

DNA methylation analysis

The SNRPN DNA methylation pattern in the normal control shows that a paternally derived unmethylated band of 4.7 kb and a maternally derived methylated band of 6.6 kb were detected for HindIII/HpaII digestion using the probe. PWS patients with 15q11-q13 deletion, mUPD, or imprinting mutation have only the maternal band, 6.6 kb. The present patient had both paternal and maternal bands, showing the normal methylation pattern (data not shown).

Discussion

We demonstrated a patient who had some clinical features of PWS. The diagnostic criteria by Holm et al. were adopted for diagnosis of PWS. These criteria have major, minor, and supportive categories, and a scoring system is used. However, these categories are made up of clinical findings except one, 15q cytogenetic or molecular abnormality. Many features of the disorder are non-specific and can be subtle or evolve over time. It is suggested that PWS is a contiguous gene syndrome, so mutation in one of these genes may show only some clinical features and may contribute to some patients.

Table 1 The results of the microsatellite markers analysis. Alleles are denoted 1–4 in descending size.

<table>
<thead>
<tr>
<th>Marker</th>
<th>Father</th>
<th>Mother</th>
<th>Patient</th>
<th>Interpretation</th>
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<tbody>
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<td>1, 2</td>
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<tr>
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<td>1, 2</td>
<td>1, 2</td>
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</tr>
<tr>
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<td>1, 2</td>
<td>1, 3</td>
<td>Biparental</td>
</tr>
<tr>
<td>D15S986</td>
<td>1, 2</td>
<td>3, 4</td>
<td>1</td>
<td>Maternal deletion</td>
</tr>
<tr>
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<td>1, 2</td>
<td>3, 4</td>
<td>1, 3</td>
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</tr>
<tr>
<td>GABRB3</td>
<td>1, 2</td>
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<tr>
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<tr>
<td>D15S127</td>
<td>1, 2</td>
<td>2, 3</td>
<td>2, 3</td>
<td>Biparental</td>
</tr>
</tbody>
</table>

Alleles are denoted 1 to 4 in descending size.

Fig. 2 Silver stain of microsatellite markers electrophoresis at D15S11, D15S986, and D15S126. F, Father; M, mother; P, patient. At D15S986, the patient has only paternal allele (closed square) and deleted from maternal allele (open or closed circle). At D15S11 and D15S126, the patient inherits biparental alleles.
being classified clinically as atypical PWS. Surprisingly, a recent study reported 15 of 90 PWS patients (16.7%) with molecular diagnosis did not meet the clinical diagnostic criteria retrospectively, proposing a lower threshold to prompt diagnostic DNA testing. The present patient had poor sucking, hypogonadism, developmental delay, hyperphagia, and esotropia, but did not have a score to meet the requirement for diagnosis. So we analyzed microsatellite markers on 15q11-q13, but he showed no deletion from the paternal allele, and did not show maternal UPD. Surprisingly, D15S986 from the maternal allele was deleted in this patient. The deletion around the maternal 15q11-q13 causes AS, but he has never demonstrated clinical findings for AS. He had normal methylation pattern about SNRPN and PW71B. The result revealed that the patient did not have an imprinting mutation. Typical PWS cases involving patients with imprinting mutation are suggested to have only a maternal methylation pattern, but recently some cases of atypical PWS have been reported to have a normal and aberrant mixed methylation pattern in which a normal pattern at SNURF-SNRPN exon1 and an abnormal pattern at D15S63 (PW71) were detected. Interestingly, those two patients had a weak maternal band and a strong paternal band at the CfoI site within PW71/exon u1A (i.e. an AS-like pattern), although they were suspected of having PWS. This may reflect the incomplete imprint spreading and suggests the existence of exception of the relationship between the clinical findings and the molecular findings. The present case showed the deletion of the very small region from the maternal allele, an AS-like pattern, though he has some clinical findings for PWS. The result indicates that the critical region for AS does not contain the region around D15S986. D15S986 is located in between the region of AS containing UBE3A and the region of PWS containing GABRB3A, GABRA5, and GABRG3. A model (Fig. 4) shows double negative regulators. In this model nearby D15S986 derived from maternal chromosome there are factors (N1) that control negatively PWS-related genes derived from paternal chromosome. In normal state the region containing D15S986 derived from maternal chromosome has a negative regulator above factors, so PWS genes derived from paternal chromosome are expressed. In contrast, in the state of micro deletion containing D15S986, N1 have negative control for PWS related genes directly, so PWS related genes are not expressed sufficiently. The clinical findings of the patient showing downslanting palpebral fissures and webbed neck are different from the typical PWS. The difference may be caused by the range of negative regulation spreading. The findings of this study underpin the importance of a positive molecular study in patients with

Fig. 3 Southern blot analysis for the SNRPN DNA methylation pattern. 4.2 kb, maternally derived pattern; 0.9 kb, paternally derived pattern; lane 1, patient; lane 2, PWS with del(15)(q11.2-q12); lane 3, AS with del(15)(q11.2-q12); lanes 4 and 5, normal control. The patient has the normal methylation pattern.

Fig. 4 (a) Double negative regulator model. N1 regulate PWS related genes negatively. The region nearby D15S986 regulates N1. (b) In the state of D15S986 deletion N1 regulate negatively PWS related genes directly.
some clinical findings of PWS, and that some symptoms for PWS need the gene of biallelic expression adjacent to the narrow region.

References


