Prader-Willi Syndrome
due to uniparental disomy in a patient
with a balanced chromosomal translocation

Gabriela Calounova 1, Drahuse Novotna 1, Martina Simandlova 1, Marketa Havlovicova 1, Alena Zumrová 2, Eduard Kocarek 1 & Zdenek Sedlacek 1

1 Institute of Biology and Medical Genetics,
2 Department of Child Neurology, Charles University Second Medical School and University Hospital Motol, Prague, Czech Republic

Correspondence to: Gabriela Calounova
Institute of Biology and Medical Genetics of Charles University Second Medical School and University Hospital Motol,
V Uvalu 84, 150 06 Prague 5, CZECH REPUBLIC
TEL: +420 224433521
FAX: +420 224433520
EMAIL: gabriela.calounova@post.cz

Submitted: September 6, 2006  Accepted: September 12, 2006

Key words: Prader-Willi syndrome; Chromosome 15; Genomic imprinting; DNA methylation; Maternal uniparental disomy; Balanced translocation.

Abstract
OBJECTIVES: In contrast to most human autosomal genes which are expressed biallelically, the expression of imprinted genes depends on the parental origin of the allele. Prader-Willi syndrome is a neurobehavioral disorder in which the expression of active paternal alleles of imprinted genes from chromosomal region 15q11-q13 is abolished by deletions, maternal uniparental disomy or imprinting defects. We report an unusual case of maternal uniparental disomy of chromosome 15 due to a balanced translocation t(8;15)(q24.1;q21.2) leading to Prader-Willi syndrome in a 3-year-old girl.

METHODS AND RESULTS: Cytogenetic investigation revealed a balanced translocation t(8;15)(q24.1;q21.2) in the patient and subsequently also in her unaffected mother. Fluorescence in situ hybridization analysis did not reveal any deletion of the PWS critical region, but methylation analysis of the SNRPN gene showed an abnormal methylation pattern indicating the absence of paternal chromosome 15. Microsatellite analysis of multiple loci and methylation-specific MLPA analysis confirmed maternal uniparental heterodisomy of chromosome 15 as the cause of PWS in the patient.

CONCLUSIONS: This example emphasizes the importance of uniparental disomy testing in pregnancies of carriers of chromosomal aberrations with participation of chromosomes carrying imprinted genes involved in human diseases.
In this report we describe a girl with PWS and apparently balanced familial reciprocal chromosomal translocation t(8;15)(q24.1;q21.2) not involving the PWS critical region 15q11-q13. A combination of several molecular genetic and cytogenetic methods showed that PWS in this patient resulted most likely from unbalanced segregation of the chromosomes involved in the translocation in a meiosis of her mother, and subsequent loss of the paternal chromosome 15 from the trisomic embryo. This finally led to maternal UPD, absence of the active paternal alleles of the critical genes, and development of PWS in the patient.

Materials and methods

The patient.

The girl was born at 40 weeks of gestation as a first child to a 31-year-old mother and a 39-year-old father. The parents were healthy and non-consanguineous. The foetal movements and liquor volume were normal. The birth weight of the girl was 2550 g (25–50th percentile) and her length was 47 cm (25th percentile). The Apgar score was 10-10-10 at 1, 5 and 10 minutes. Soon after the birth a congenital heart defect (multiple apical ventricular septal defect) and malrotation of the right kidney were diagnosed. Neurological examination revealed a mild central hypotonia and decreased reflexes. The developmental milestones of the girl were delayed. She walked without support from the age of 18 months, and at that time she also said her first words. Hyperphagia and obesity evolved at the age of 3 years. Her weight was 23 kg (above the 95th percentile), her height was 96 cm (50–70th percentile), and she exhibited other characteristic features of PWS – almond shaped eyes, down slanting palpebral fissures and a narrow bifrontal diameter. She was blond haired but with no other areas of hypopigmentation. Her cognitive development was only mildly delayed, and she showed good language and social skills.

Classical cytogenetic and fluorescence in situ hybridization (FISH) analysis.

Chromosomal aberrations in peripheral blood lymphocytes of the patient and her parents were analysed using standard cytogenetic techniques. Fine mapping of chromosome 15 was performed using FISH. Locus-specific probes for the PWS critical region (D15S10, UBE3A, SNRPN), chromosome 15 centromeric probe D15Z1 and 15q subtelomeric probe D15S936 were hybridized to chromosomes of the patient and her mother according to the protocols of the manufacturers (Cytocell, Vysis).

Methylation analysis.

Genomic DNA was isolated from blood lymphocytes of the patient and her parents using a commercial kit (Puregene, Gentra), and the methylation status of genes in the PWS critical region was tested by two independent methods. Firstly, the SNRPN gene was analysed by meth-
ylation-specific PCR (MS-PCR) based on allele-specific amplification of DNA treated by sodium bisulphite (CpGenome DNA Modification Kit, Chemicon) [29] and 2% agarose gel electrophoresis of the resulting fragments. Secondly, the methylation of the SNRPN and NDN gene promoters and the copy number of these two and several other chromosome 15 genes was analysed by methylation-specific multiplex ligation-dependent probe amplification (MS-MLPA) [17] using the ME028 PWS/AS Kit according to the instructions of the manufacturer (MRC-Holland). Here the ligation of the probes was combined with digestion of the genomic DNA-probe hybrids by methylation-sensitive restriction endonuclease Hha I (Promega) prior to DNA amplification. In total the assay included 25 probes specific for most of the genes in the PWS region, and 15 probes for genes located elsewhere. Five probes contained a Hha I restriction site. The resulting DNA fragments were resolved using a capillary sequencer (ABI PRISM 3100 Avant, Applied Biosystems).

Microsatellite analysis.
In total 22 highly polymorphic microsatellite DNA markers were used for the assessment of the copy number and parental origin of multiple chromosome 15 loci (Table 1). The markers were located in the PWS critical region, in the deletion breakpoints regions, and distal from 15q11-q13 (CYP19, D15S642). Fluorescently labelled forward primers were employed and the PCR products were separated on 6% PAGE / 7M urea on the Gel Scan 2000 (Corbett Research).

Results

Cytogenetic studies and FISH.
Conventional chromosome analysis showed a balanced reciprocal translocation t(8;15)(q24.1;q21.2) in the patient. The translocation breakpoint was located far distal to the PWS critical region 15q11-q13 (Figure 1). The unaffected mother of the patient carried the same balanced translocation, and the father had a normal karyotype. All three probes specific for the PWS critical region used for the FISH analysis showed normal hybridization signals on both the normal and the translocated chromosome 15 of the patient (Data not shown). These results excluded a deletion as the cause of PWS in the patient.

Methylation analysis.
MS-PCR can detect deletions, UPD and imprinting defects associated with PWS. In a normal individual, the assay yields a 321 bp DNA fragment from the methylated maternal allele and a 213 bp fragment from the non-methylated paternal allele of the SNRPN gene [29]. Both the maternal and the paternal bands were observed in the patient’s mother and father. However, the DNA from the patient showed only the maternal 321 bp band (Figure 2), confirming the absence of the non-methylated paternal SNRPN allele and the diagnosis of PWS in the patient.

The MS-MLPA analysis indicated a normal copy number at all 25 sites tested in the PWS region. However, abnormal methylation pattern (100% instead of 50% methylation) could be detected at all five methylation-sensitive test sites in the SNRPN and NDN genes (Figure 3). Thus this assay confirmed independently an absence of non-methylated paternal material without loss of the

Figure 1. The cytogenetic analysis. Partial GTG-banded karyotype of the patient illustrates chromosomes 15 and 8 undergoing a balanced reciprocal translocation t(8;15)(q24.1;q21.2). Translocation breakpoints (arrows) and the PWS critical region (black rectangle) are indicated. Chromosome schematics are from www.pathology.washington.edu/research/cytopages/idiograms/human/.
normal copy number in the critical region due to the presence of two methylated maternal alleles.

**Microsatellite analysis.**

Of the 22 chromosome 15 markers used in the analysis, 13 markers were informative, and all showed an absence of the paternal allele in the patient. Of these 13 markers, seven (4 inside the critical region, 2 in the centromeric breakpoint region, and 1 distal to the 15q11-q13 region) were fully informative, and indicated the presence of two different maternal alleles in the patient (Table 1). These results confirmed maternal uniparental heterodisomy as the cause of PWS in the patient.

**Discussion**

The clinical symptoms observed in our patient were strongly suggestive of the Prader-Willi syndrome. The newborn girl was hypotonic with mild dysmorphism and a heart defect, and typical obesity evolved at the age of 3 years. Her cognitive development was mildly delayed with characteristic facial features of PWS. Studies of patients with PWS show that individuals with paternal deletions have a typical PWS phenotype [10,9] while patients with maternal UPD may have a milder phenotype with better cognitive functions [19,2]. The clinical findings in our patient fit to these observations.

To confirm the diagnosis of PWS and to allow sound genetic counselling in the family we have undertaken a complex analysis of the molecular mechanisms, which may have led to the development of this disorder in the patient. Cytogenetic analysis showed a balanced reciprocal translocation t(8;15) not involving the PWS critical region 15q11-q13. The patient inherited the translocation from her unaffected mother. Although maternal UPD associated with the translocation represented the most likely explanation of the phenotype of the patient, any of the two remaining major causes of PWS, a deletion or an imprinting defect, could not be a priori excluded as the causative mechanism. The patient had very blond hair and a pale skin, and these traits may point in PWS patients to deletions [2]. Also, several studies described de novo deletions leading to PWS in patients who had a parent with a balanced translocation involving chromosome 15 with breakpoints either inside or outside the PWS region, and the authors speculated that these translocations could predispose to further chromosome 15 rearrangements [28,23,11,5]. These results as well as
increasing knowledge of very high complexity behind some apparently simple cytogenetic rearrangements [8,3] prompted us to perform deletion analysis, methylation analysis, and genotyping of chromosome 15 markers in the family. The FISH analysis excluded a deletion of the critical region as a cause of PWS in the patient, and the methylation analysis confirmed the absence of the active non-methylated paternal allele in the genome of the patient. The microsatellite analyses showed convincingly maternal UPD of chromosome 15 at 13 loci tested, and the genotype at 7 of these loci clearly indicated maternal uniparental heterodisomy.

Maternal UPD of chromosome 15 is responsible for approximately 20–25% of PWS cases [16]. UPD results most often from meiotic non-disjunction followed by postzygotic correction either by reduction of a trisomic

<table>
<thead>
<tr>
<th>Microsatellite DNA marker</th>
<th>Genotyping results</th>
<th>Conclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Name</td>
<td>Position (Mb)*</td>
<td>Mother</td>
</tr>
<tr>
<td>D15S541</td>
<td>20.5</td>
<td>1 2</td>
</tr>
<tr>
<td>D15S542</td>
<td>20.5</td>
<td>3 4</td>
</tr>
<tr>
<td>D15S1035</td>
<td>20.5</td>
<td>3 4</td>
</tr>
<tr>
<td>D15S543</td>
<td>21.2</td>
<td>2 2</td>
</tr>
<tr>
<td>D15S111</td>
<td>21.6</td>
<td>1 1</td>
</tr>
<tr>
<td>D15S122</td>
<td>23.2</td>
<td>2 4</td>
</tr>
<tr>
<td>D15S10</td>
<td>23.2</td>
<td>1 2</td>
</tr>
<tr>
<td>D15S210</td>
<td>23.3</td>
<td>1 3</td>
</tr>
<tr>
<td>D15S113 I</td>
<td>23.8</td>
<td>2 2</td>
</tr>
<tr>
<td>D15S113 II</td>
<td>23.8</td>
<td>3 3</td>
</tr>
<tr>
<td>GABRB3</td>
<td>24.3</td>
<td>3 4</td>
</tr>
<tr>
<td>D15S97</td>
<td>24.4</td>
<td>3 3</td>
</tr>
<tr>
<td>D15S1364</td>
<td>24.5</td>
<td>2 2</td>
</tr>
<tr>
<td>GABRA5</td>
<td>24.8</td>
<td>1 2</td>
</tr>
<tr>
<td>D15S156</td>
<td>25.6</td>
<td>1 2</td>
</tr>
<tr>
<td>D15S217</td>
<td>25.7</td>
<td>1 1</td>
</tr>
<tr>
<td>D15S1019</td>
<td>27.4</td>
<td>1 1</td>
</tr>
<tr>
<td>D15S165</td>
<td>29.0</td>
<td>2 2</td>
</tr>
<tr>
<td>D15S144</td>
<td>31.4</td>
<td>1 3</td>
</tr>
<tr>
<td>D15S518</td>
<td>40.2</td>
<td>3 4</td>
</tr>
<tr>
<td>CYP19</td>
<td>49.3</td>
<td>2 4</td>
</tr>
<tr>
<td>D15S642</td>
<td>100.2</td>
<td>2 2</td>
</tr>
</tbody>
</table>

* Position on chromosome 15 according to Human Genome March 2006 Assembly (http://genome.ucsc.edu)
zygote or by correction of a monosomic zygote to a disomic one [27,6]. The most likely mechanism of the maternal uniparental heterodisomy in our patient was a 3:1 segregation of the translocation in her mother resulting in an ovum with one normal chromosome 15, and both the rearranged chromosomes der(15) and der(8). Fusion of this ovum with a normal paternal gamete resulted in a zygote trisomic for chromosome 15. Finally, a postzygotic correction (rescue) of this trisomic embryo via a chance removal of the single paternal chromosome 15 led to the restoration of the balanced karyotype observed in the mother of the patient, but with both the normal chromosome 15 and the der(15) of maternal origin (Figure 4).

The risk of non-disjunction for any successive pregnancy in this family is high. Compared to normal individuals, carriers of familial translocations have a highly increased likelihood of aberrant chromosome segregation. Preimplantation genetic diagnosis in a female carrier of a balanced reciprocal translocation t(8;13)(q24.1;q22) [21] can be used to illustrate this risk. Analysis of the rearranged chromosomes in nine first polar bodies identified one 2:2 alternate segregation, three 2:2 alternate or adjacent 1 segregation involving a recombinant chromosome 13 (with one normal and one derivative chromatid), one 2:2 adjacent 2 segregation, and four 3:1 segregations [21]. The only segregation pattern producing normal or balanced gametes is the 2:2 alternate pattern, in which the two normal chromosomes segregate to one pole, whereas the two derivatives go to the other one. Of the several types of unbalanced chromosomal constitutions in gametes produced in translocation carriers, some can be corrected postzygotically but with a high risk of UPD.

In accord with this concept, PWS caused by UPD was observed in the progeny of several carriers of Robertsonian translocations involving chromosome 15 [16,25,26]. Familial reciprocal translocations involving chromosome 15 leading to UPD have been reported in two families with PWS and two with Angelman syndrome (AS, where the expression of active maternal alleles of imprinted genes from 15q11-q13 is lacking) [23,20,7,24]. However, the genetic scenarios in these families were different to that in the current case. The family of Park et al. carried a translocation t(3;15)(p25;q11.2) and the UPD in the PWS patient originated most likely from fusion of a sperm carrying a normal chromosome 3 and a der(15) with an ovum disomic for chromosome 15. The patient carried a small supernumerary der(15) chromosome and his karyotype was not balanced [20]. Flori et al. described a prenatally diagnosed PWS case with maternal UPD 15 in consanguineous parents both carrying a translocation t(14;15)(q11;q13) [7]. Smeets et al. documented a family with a familial unbalanced translocation t(6;15)(p25.3;q11.1) in which one child with AS inherited two copies of chromosome 15q11-q13 from the father [23]. Another case of AS resulted from paternal UPD due to a familial unbalanced translocation t(8;15)(p12.3;q11) [24]. In addition to chromosome 15 UPD, both of the latter patients had also losses of small portions of the respective chromosomes involved in the translocations.

Bearing in mind the phenomenon of genomic imprinting, pregnancies of carriers of familial Robertsonian or reciprocal translocations undergoing prenatal diagnosis as well as cases with mosaicism for a trisomy or a monosomy (which may point to a postzygotic correction) should be tested not only for the respective chromosome aberration but also for UPD. In addition to cases of PWS and AS described above and in our report, maternal UPD of chromosome 7 leading to Silver-Russell syndrome was identified in a child from a family with translocation t(7;16)(q21;q24) [4]. The UPD testing should therefore not be limited to chromosome 15 abnormalities but should be performed also when other chromosomes known to carry imprinted genes playing a role in human diseases (e.g. chromosomes 7, 11, 14, 20, and possibly 2, 6, 16) are involved [14,27].

Acknowledgements

We thank Janka Rydlova for technical assistance and Marta Kopeckova for helpful discussions. The work was supported by grants GAUK 61/2004 and MZO 00064203.

REFERENCES

9. Gunay-Aygun M, Schwartz S, Heeger S, O Riordan MA, Cassidy SB. The changing purpose of Prader-Willi syndrome: Clinical di-