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.**

Neuroendocrinol Lett 2006; **27**(5):579–585 **PMID:** 17159828 NEL270506A04

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.

ORIGINAL ARTICLE

Abbreviations

PWS	– Prader-Willi syndrome
OMIM	– database Online Mendelian Inheritance in Man
UPD	– uniparental disomy
IC	– imprinting centre
FISH	– fluorescence in situ hybridization
MS-PCR	– methylation-specific PCR
MS-MLPA	– methylation-specific multiplex ligation-dependent probe amplification
SNRPN gene	– small nuclear ribonucleoprotein polypeptide N gene
NDN gene	– necdin gene
PAGE	– polyacrylamid gel electrophoresis

Introduction

Most autosomal genes in the human genome are biallelically expressed. However, a small number of imprinted genes (the current estimates are around 100) are expressed only from one chromosome depending on its parental origin, and the expression of the second allele of these genes is silenced [14]. Aberrant expression of imprinted genes causes several disorders in human [14]. The most frequent is the Prader-Willi syndrome (PWS, OMIM 176270), a neurogenetic disorder affecting one in about 10–15 000 live births [10,9]. PWS is characterized by diminished fetal activity, obesity, muscular hypotonia, mental retardation, short stature, hypogonadotropic hypogonadism, and small hands and feet.

PWS is caused by the lack of expression of active paternal alleles of several imprinted genes from chromosomal region 15q11-q13 due to one of several genetic mechanisms [15]. The majority of patients (about 65–75% of all cases) have de novo deletions spanning 4 to 4.5 Mb of the critical region on the paternal chromosome 15 [12,13]. The second most common cause of PWS (20–25% of cases) is the presence of two copies of the critical region, but both of them of maternal origin (maternal uniparental disomy – UPD). Patients in the third group (about 5% of cases) have an imprinting defect – a submicroscopic deletion of an element termed the imprinting centre (IC), or just an abnormal epigenotype without any detectable mutation on the nucleotide sequence level [1,18]. Finally, several individuals have been reported with de novo balanced reciprocal chromosomal translocations involving the paternal copy of 15q11-q13 where the PWS phenotype was likely to be the result of disruption of the SNRPN gene locus [22].

The genetic mechanisms underlying the syndrome may influence the spectrum and severity of developmental and behavioural problems and mental impairment characteristic for the syndrome, as well as the individual symptoms including infantile hypotonia, genital hypoplasia, neonatal feeding difficulties followed by hyperphagia leading to profound obesity around two years of age, mental retardation, small hands and feet, and hypopigmentation [10,9,19,2]. Although the majority of cases of PWS are sporadic, the elucidation of the causative genetic mechanism is very important for the risk assessment and genetic counselling in affected families.

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

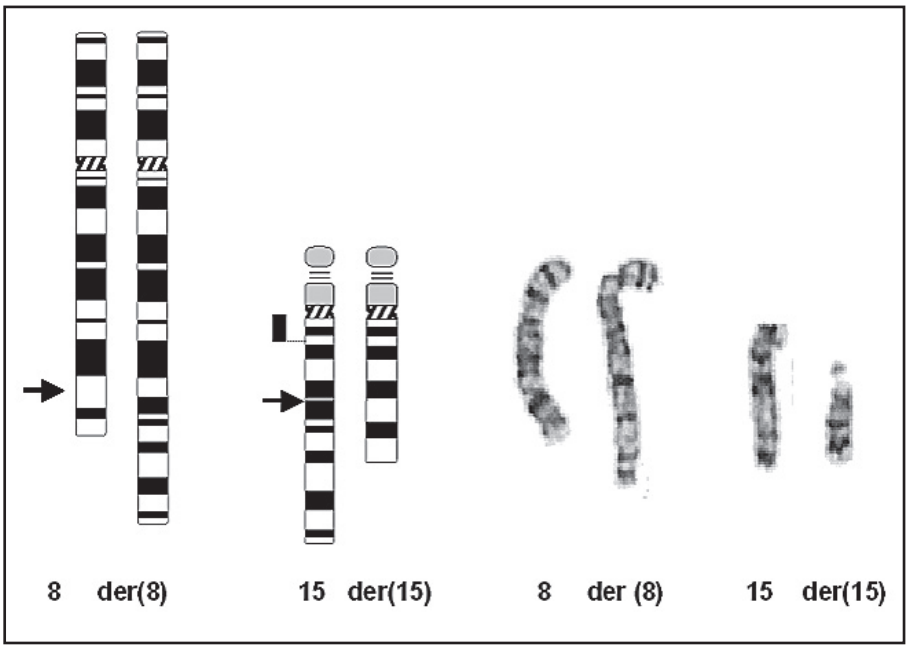

Figure 1. The cytogenetic analysis. Partial GTG-banded karyotype of the patient illustrates chromosomes 15 and 8 undergoing a balanced reciprocal translocation t(8;15)(q24.1;q21.2). Translocation breakpoints (arrows) and the PWS critical region (black rectangle) are indicated. Chromosome schematics are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

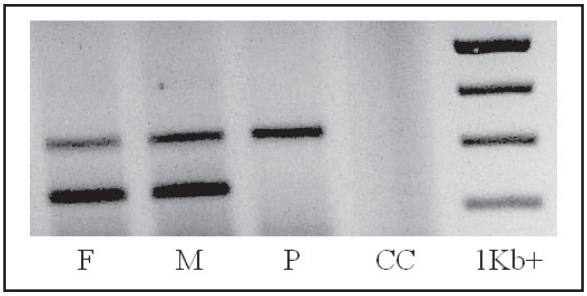

Figure 2. Methylation-specific PCR. The 321 bp band represents a methylated maternal allele, the 213 bp band represents a non-methylated paternal allele. The paternal allele is clearly absent in the patient while her parents show a normal pattern.
F-father, M-mother, P-patient, CC-contamination control,
1Kb+ - DNA length standard (Invitrogen).

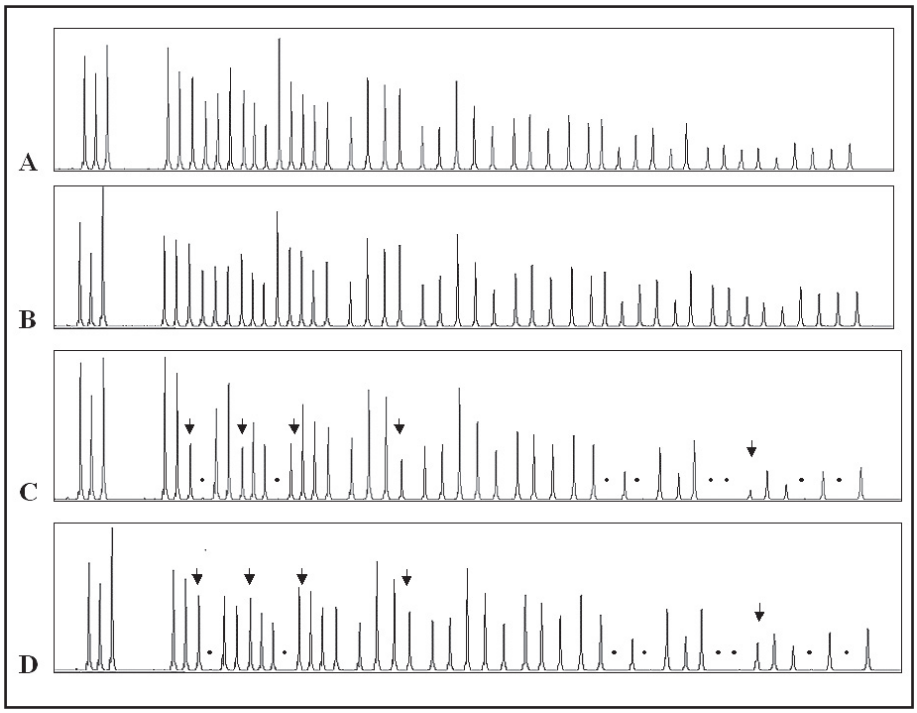

Figure 3. Methylation-specific MLPA. Peak profiles of a normal control (A, C) and the patient (B, D) on undigested DNA-probe hybrids (A, B) and Hha I digested hybrids (C, D). Due to the presence of two methylated maternal alleles at all five differently methylated sites tested in the 15q11-q13 region (arrows) the patient shows no differences in peak areas of the signals between the Hha I digested (D) and undigested DNA (B).
The normal control DNA with one methylated allele and one non-methylated allele shows a 50% reduction of the signals from the digested DNA (C) compared to the undigested DNA (A).
Total disappearance of specific MS-MLPA signals (dots) indicates a complete Hha I digestion.

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

Table 1: The genotypes identified by microsatellite analysis.

Microsatellite DNA marker		Genotyping results			Conclusion
Name	Position (Mb)*	Mother	Proband	Father	
D15S541	20.5	1 2	1 2	1 1	Not informative
D15S542	20.5	3 4	3 4	1 2	Maternal hetero-UPD
D15S1035	20.5	3 4	3 4	1 2	Maternal hetero-UPD
D15S543	21.2	2 2	2 2	1 3	Maternal UPD
D15S11	21.6	1 1	1 1	1 1	Not informative
D15S122	23,2	2 4	2 4	1 3	Maternal hetero-UPD
D15S10	23,2	1 2	1 2	1 1	Not informative
D15S210	23,3	1 3	1 3	2 4	Maternal hetero-UPD
D15S113 I	23,8	2 2	2 2	1 1	Maternal UPD
D15S113 II	23,8	3 3	3 3	1 2	Maternal UPD
GABRB3	24,3	3 4	3 4	1 2	Maternal hetero-UPD
D15S97	24,4	3 3	3 3	1 2	Maternal UPD
D15S1364	24,5	2 2	2 2	1 1	Maternal UPD
GABRA5	24,8	1 2	1 2	2 2	Not informative
D15S156	25,6	1 2	1 2	1 1	Not informative
D15S217	25,7	1 1	1 1	2 2	Maternal UPD
D15S1019	27,4	1 1	1 1	1 1	Not informative
D15S165	29.0	2 2	2 2	1 2	Not informative
D15S144	31.4	1 3	1 3	2 3	Not informative
D15S518	40,2	3 4	3 4	1 2	Maternal hetero-UPD
CYP19	49.3	2 4	2 4	1 3	Maternal hetero-UPD
D15S642	100.2	2 2	2 2	1 2	Not informative

* Position on chromosome 15 according to Human Genome March 2006 Assembly (http://genome.ucsc.edu)

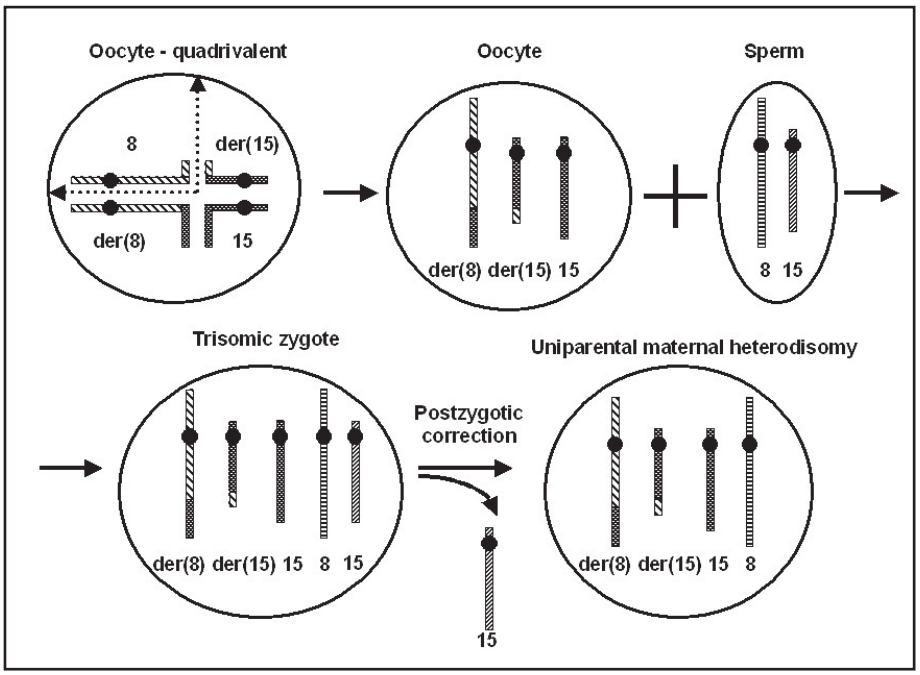

Figure 4. The most likely scenario of events leading to PWS in the patient. See text for details.

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

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.

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