

Prader-Willi syndrome and a deletion/duplication within the 15q11-q13 region

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Prader-Willi syndrome (PWS) is characterised by infantile hypotonia, feeding difficulties, hypogonadism, small hands and feet, mental deficiency, obesity in early childhood, a particular facial appearance, and a paternally derived 15q11-q13 deletion (approximately 4 million bp in size) in about 70% of subjects, maternal disomy 15 (both 15s from the mother) in 25% of subjects, or an imprinting mutation in 2–5% of subjects.^{1–4} PWS syndrome is considered to be the most common genetic cause of marked obesity in humans.¹

Two breakpoint clusters have been reported centromeric to locus ZNF127 with the most proximal breakpoint (BP1) accounting for 44% of cytogenetic deletions while 56% of deletions occur at the second proximal breakpoint (BP2).^{5–6} The second breakpoint (BP2) lies between loci D15S541/S542 and D15S543 and breakpoint BP1 is proximal to D15S541/S542.^{5–6} A third breakpoint (BP3) is distally located within the 15q11-q13 region and mapped telomeric to the P locus (involved in hypopigmentation) in nearly all deletion subjects studied.^{5–13} The apparent genetic instability in the 15q11-q13 region may be attributed to DNA sequences identified in low copy repeats in the vicinity of the common breakpoints occurring in patients with PWS. These END repeats are derived from large genomic duplications of a novel gene (*HERC2*).^{8–12} The END repeats flanking the 15q11-q13 region may be involved with inter- and intrachromosomal misalignment and homologous recombination resulting in the common PWS deletion and facilitated by active transcription of the END repeats in male and female gametogenesis.^{12–14}

Duplication, triplication, and tetrasomy of the 15q11-q13 region have been reported with varying degrees of clinical manifestation. Generally, duplications of maternal origin have been associated with developmental delay and autistic behaviour.^{15–18} Paternal duplications have no apparent impact on the phenotype although a patient reported by Mohandas *et al*¹⁹ with an interstitial duplication of proximal 15q of paternal origin had non-specific developmental delay and partial agenesis of the corpus callosum. In addition, Ungaro *et al*⁶ reported molecular characterisation of four subjects presenting with mild to severe mental retardation and features of PWS or Angelman syndrome along with intrachromosomal triplications of the chromosome region 15q11-q14.

Here, we present a patient with the typical 15q11-q13 deletion seen in PWS who also carried a familial duplication of centromerically located 15q11-q13 loci. The paternally derived deletion of 15q11-q13 occurred at breakpoints BP2 and BP3 and the paternal duplication was centromeric to breakpoint BP2.

CASE REPORT

Our patient was born at 30 weeks' gestation by vertex vaginal delivery. She weighed 1531 g and was 40 cm long. Bleeding problems were reported during the pregnancy but no admission to hospital was required. Decreased fetal activity was noted. Hypotonia and a poor suck reflex with feeding difficulties were observed shortly after birth. Chromosome studies showed the typical chromosome 15q11-q13 deletion confirmed by fluorescence in situ hybridisation using probes

for *SNRPN*, D15S11 and *GABRB3*. In addition, polymerase chain reaction (PCR) methylation testing was diagnostic for PWS. Historically, she had the typical features seen in PWS including a narrow forehead, small upturned nose, downturned mouth, hypotonia, small hands and feet, obesity in early childhood, learning and behavioural problems, and skin picking. She also had hypopigmentation in relation to other family members, typically seen in those with a 15q11-q13 deletion.^{13–20} She has not been diabetic and had normal thyroid function tests in the past although she had a long history of obesity. She has had a history of unusual eating patterns with hyperphagia and food foraging leading to early childhood obesity. The refrigerator and kitchen cabinets are locked in the home environment. She has not had sleep apnoea but does experience drowsiness. At 17 years of age she weighed 98.9 kg (>>97th centile) and was 149.9 cm (3rd centile) tall. She has not been treated with psychotropic medication although behavioural problems including skin picking were present in early childhood. She had developmental delay and mild mental retardation. She had a history of reading and maths difficulties requiring special education and speech therapy. She enjoys swimming but does not like to cycle, walk, or dance. She enjoys working with crafts and jigsaw puzzles.

Microsatellite analysis with PCR using 19 short tandem repeats from the 15q11-q13 region showed a paternally derived deletion of several informative loci (for example, D15S817, D15S63, D15S210, *GABRA5*, D15S822) supporting breakpoints at BP2 and BP3. Interestingly, she had three alleles at D15S541, D15S542, and D15S1035, which are centromeric to breakpoint BP2 within the 15q11-q13 region, indicating a duplication of these loci (table 1). The same duplication was shared by her phenotypically normal father and uncles by PCR analysis but not by the proband's two unaffected female sibs. No other person tested in the family showed the deletion or duplication (table 1).

DISCUSSION

Our report illustrates the clinical and molecular findings of a subject with classical features of PWS with the typical deletion of 15q11-q13 seen in 70% of PWS subjects. In addition, she had a familial duplication of centromerically located 15q11-q13 loci inherited from her father. The clinical presentation is described in the text and the molecular findings summarised in table 1 with microsatellite data shown in fig 1. Our laboratory has analysed DNA microsatellite data from the 15q11-q13 region including D15S541, D15S542, D15S543, and D15S1035 loci in over 50 PWS families including the parents. However, this was the only family we identified with a duplication of loci centromerically located within the 15q11-q13 region. To our knowledge, this PWS subject is the first reported with the typical 15q11-q13 deletion and a duplication of genetic loci inherited from the father centromeric to breakpoint BP2.

Interstitial duplications of 15q11-q13 of maternal origin have been identified in patients with developmental delay and learning/behavioural problems while subjects with paternally derived duplications generally have a normal phenotype as seen in our unaffected father and the proband's two paternal uncles. However, a patient reported by Mohandas *et al*¹⁹ had an

Table 1 PCR and FISH data in linear order from the 15q11-q13 region

	D15S1035	D15S541	D15S542	D15S543	D15S11	D15S817	D15S63	D15S128	MN1	SNRPN	D15S122	D15S210	D15S113	GABRB3	GABRA5	D15S822	D15S156	D15S219	D15S217	D15S165
Mother	1,4	2,4	2,3	2,2	2,2	2,4	2,3	3,3	1,1	1,1	3,4	1,3	1,3	1,2	2,2	1,3	1,2	1,2	1,2	1,3
PWS proband	3,4,5*	2,3,4*	3,4,5*	2,2 or 2,-	2(-)	4†	2†	3†	1‡	(-)	4†	3‡	3(-)	2†	2†	3,3 or 3,-	2,2 or 2,-	1,1 or 1,-	2,3‡	2,3‡
Father	1,3,5	1,2,3	1,4,5	1,2	1,2	1,3	1,3	1,2	1,2	2,2	1,2	2,3	2,3	3,3	1,3	2,3	2,2	1,1	1,1	2,3
Sib 1	1,1			1,2																
Sib 2	1,1			1,2																
Paternal uncle 1	1,3,5			1,2																
Paternal uncle 2	2,3,5			1,2																

*Paternal duplication status; †paternal deletion status; ‡deletion inferred by proximal and distal loci status; §biparental (normal) inheritance. (-) = deletion status determined by FISH.

interstitial duplication of proximal 15q containing the Prader-Willi syndrome/Angelman syndrome region (for example, *GABRB3* locus) of paternal origin and an abnormal phenotype. The duplication they reported was larger than in our patient. They reported that their patient's genetic findings were consistent with the origin of the duplication from unequal crossing over between the two chromosome 15 homologues from the father.

Our family lends support with anecdotal and laboratory data that a parent (father) with a genomic duplication within the 15q11-q13 region may predispose to unequal crossing over in meiosis leading to a deletion event within the region and therefore PWS. Additional studies are needed to determine if the apparent duplication of the proximal 15q loci seen in our PWS proband and her father and paternal uncles without recognisable clinical findings is a common polymorphism or impacts on meiosis and crossing over events leading to a deletion. In addition, a study to compare the frequency of this molecular finding in fathers of PWS children compared to fathers in the general population would be of importance to address this observation seen in our PWS family.

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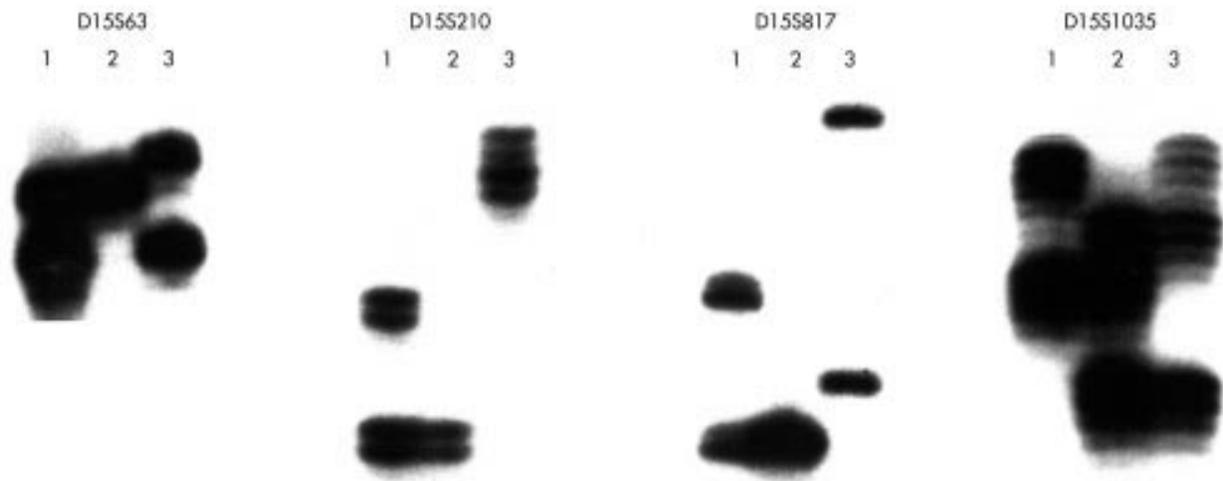


Figure 1 Polymerase chain reaction (PCR) from genomic DNA of several informative loci (D15S63, D15S210, and D15S817) from the 15q11-q13 region showing only one allele for the PWS patient (lane 2) consistent with a paternal deletion. PCR result of the D15S1035 locus from the centromeric end of the 15q11-q13 region shows three alleles, consistent with a paternal duplication. Lanes 1, 2, and 3 represent the mother, PWS subject, and father, respectively.

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The frequency of mtDNA 8994 polymorphism and detection of the NARP 8993 mutation

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The highly polymorphic nature of the mitochondrial genome (mtDNA) has proved valuable to the population geneticist, but can cause serious problems in the identification of disease causing mutations.

A T→C or T→G transition at nt 8993 in human mtDNA is associated with an array of clinical phenotypes including Neuropathy, Ataxia and Retinitis Pigmentosa (NARP)¹ and Leigh's syndrome.² Conventionally, it is detected by polymerase chain reaction (PCR) amplification of the region containing the mutant sequence followed by digestion with restriction enzymes *HpaII* or *HpaI* (recognition sequence c↓cgg) which recognise both sequence changes.¹ The presence of either mutation results in the PCR product being cut into two fragments (343 bp and 206 bp), which can be separated and identified by agarose gel electrophoresis.

A polymorphic G→A transition in the adjacent base (nt 8994) abolishes the recognition site for the relevant restriction enzymes and, hence, in patients who also have the NARP 8993 mutation can result in a false negative diagnosis.

As a consequence, a method has been developed to detect the 8994 polymorphism using the restriction enzyme *HaeIII* applied to the same undigested PCR product used for detecting the 8993 mutation, which is in use by some laboratories who test for the 8993 mutation.³ Patients without the polymorphism have three *HaeIII* restriction sites in the amplified region resulting in four fragments (190 bp, 174 bp, 156 bp, 31 bp), while those with the polymorphism have one of these sites abolished giving rise to three fragments (190 bp, 187 bp, 174 bp). Under the gel electrophoresis conditions used (2% agarose with ethidium bromide staining), the 190 bp and 187 bp fragments run as a single band and the 31 bp fragment is usually not visible as it runs off the gel. Thus, in the presence of the 8994 polymorphism two fragments are seen, while in its absence three fragments are detected.

To exclude the NARP 8993 mutation, we routinely test all patients for both the 8994 and 8993 mutations. If the 8994 polymorphism is detected, the PCR product is sequenced to exclude the 8993 mutation.