

SHORT REPORT

Highly restricted deletion of the SNORD116 region is implicated in Prader–Willi Syndrome

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The *SNORD116* locus lies in the 15q11-13 region of paternally expressed genes implicated in Prader–Willi Syndrome (PWS), a complex disease accompanied by obesity and severe neurobehavioural disturbances. Cases of PWS patients with a deletion encompassing the *SNORD116* gene cluster, but preserving the expression of flanking genes, have been described. We report a 23-year-old woman who presented clinical criteria of PWS, including the behavioural and nutritional features, obesity, developmental delay and endocrine dysfunctions with hyperghrelinemia. We found a paternally transmitted highly restricted deletion of the *SNORD116* gene cluster, the shortest described to date (118 kb). This deletion was also present in the father. This finding in a human case strongly supports the current hypothesis that lack of the paternal *SNORD116* gene cluster has a determinant role in the pathogenesis of PWS. Moreover, targeted analysis of the *SNORD116* gene cluster, complementary to *SNRPN* methylation analysis, should be carried out in subjects with a phenotype suggestive of PWS.

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INTRODUCTION

Prader–Willi Syndrome (PWS) is a neurodevelopmental disorder caused by the lack of expression of paternal alleles in 15q11-13.^{1,2} The PWS phenotype includes neonatal hypotonia, early hyperphagia, morbid obesity, short stature, hypogonadism, cognitive impairment, and behavioural and psychiatric problems. Molecular mechanisms including large deletions, maternal uniparental disomy or imprinting defects explain 98% of cases which are easily diagnosed with a *SNRPN* methylation analysis. The study of rare cases resulting from reciprocal translocations and atypical 15q11q12 microdeletions without methylation abnormalities has defined a critical region containing the functional PWS gene locus.³ This minimal region contains several snoRNAs gene clusters including *SNORD116* and *SNORD115*. Mice lacking the *SNORD116* orthologue display a partial PWS phenotype.^{4,5} However, so far, all reported clinical cases of limited deletion of the *SNORD116* cluster associated with PWS have also involved adjacent genes: *SNURF-SNRPN* or *SNORD115*.^{6–9} We report the first case of a patient with the highly typical features of PWS who presented a restricted deletion of the *SNORD116* region which did not affect the expression of *SNURF-SNRPN* and did not delete any portion of the *SNORD115* locus.

Patient

A girl was born after 35 weeks gestation with appropriate weight (2780 g), length (48 cm) and cranial perimeter (35 cm). Because of severe neonatal hypotonia and poor suck, she required exclusive tube

feeding during the first 2 weeks of life and hospital care for 1 month. She had mild developmental delay and walked at 18 months. She had an orthoptic treatment for a strabism. Excessive aggressiveness and stubbornness were observed at 30 months. She displayed poor abstraction skills and difficulties in coping with stress and frustrations, with frequent temper tantrums. She gained excessive weight after 18 months and became obese by the age of 3 (Supplementary Figure 1Sa). Strict control of food access allowed BMI stabilisation until the age of 12. She presented frequent skin picking. Growth hormone deficiency (GHD) was diagnosed at age 9 years with low IGF-1 level (Table 1), but GH treatment was not indicated as her height was above –2 SDS. GHD was again documented at age 12 years; GH treatment was started and discontinued after 6 months by the parents. L-thyroxin was given from age 10 to 13 years due to central hypothyroidism. At age 12, the GnRH test suggested central hypogonadism. At age 16, incomplete pubertal development with no menarche required substitution with oestrogen and progesterone. The patient did not accept to perform an IQ test; however, she had a mild impairment of cognitive functions. Nevertheless she remained in mainstream education until secondary school. Adult height was –1.5 SDS (Supplementary Figure 1Sb).

The patient was 23 when admitted to our PWS reference centre. Her phenotype suggested PWS with morphologic features (narrow forehead, thin and down-turned upper lip, hypopigmented skin, acromicria) (Figure 1). She had thick saliva and presented skin picking (visible in Figure 1). Abnormal sleep-wakefulness scoring and

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excessive daytime sleepiness were noted with an apnea-hypopnea index score of 3 per hour, a mild level. She had hyperphagia, temper tantrums and difficulties in organisation of daily life. Strict control of food access had allowed stabilisation of BMI at 31 kg/m² (Supplementary Figure 1Sa). GHD with low IGF-1, hypogonadism and hyperghrelinemia¹⁰ were documented (Table 1). The mother (height 168 cm, weight 55 kg, BMI 19.5 kg/m²) had a history of four miscarriages associated with positive anti-nuclear antibodies. The father (height 168 cm, weight 80 kg, BMI 28.3 kg/m²) was healthy; his brother and his parents, deceased, were otherwise asymptomatic. The couple previously had a preterm baby girl who died at the fourth day of life with severe hypotonia.

METHODS

All subjects agreed to participate and gave their signed informed consent for genetic testing. Methods are described in Supplementary Methods.

RESULTS

Methyl-specific PCR at the *SNURF-SNRPN* locus was normal in the proband. As the phenotype was highly suggestive of PWS (Figure 1), we performed quantitative multiplex PCR of short fluorescent fragments (QMPSF) analysis of 15q11q12 in order to detect atypical deletions in the 15q11.2 region (Figure 2a). Analysis of fragments revealed 50% reduction of a unique marker consistent with a heterozygous deletion in the *SNORD116* region (Supplementary Figure 2S). We determined the boundaries of the deletion using specifically designed 15q oligonucleotide-based array comparative genomic hybridisation (array-CGH). We found negative hybridisation of 333 SNP probes encompassing a segment of 116.5 kb (Figure 2b). PCR analysis and sequencing identified breakpoints at positions g.25257217 and g.25375376 based on the UCSC hg19 genome assembly (size of the deletion: 118 159 bp). Two perfectly homologous six-bp short sequences flanked the deleted region, suggesting a breakage mechanism by microhomology-mediated end joining (Figure 2c). The paternal origin of the deletion was demonstrated by a PCR assay directed to the breakpoint regions (Figure 2d). The deletion encompassed the *SNORD109A* gene, the complete *SNORD116* cluster 1–29 and the four non-coding exons of the IPW transcript (Figure 2a). Expression of *SNRPN* and *SNORD116* transcripts was studied by RT-PCR in lymphocytes and fibroblasts from the proband and her parents (Figures 2e and f). *SNRPN* was normally expressed (Figure 2e). Conversely, *SNORD116* expression was detected in both parents but was absent in the proband (Figure 2f). From RT-quantitative PCR analysis in fibroblasts, the

expression of *SNRPN* and *NECDIN* genes was abolished in patients with a type 1 PWS deletion,² but did not differ between control subjects and the proband (Supplementary Figure 4S). The genotype



Figure 1 Photography of the proband at 23 years.

Table 1 Endocrine evaluations at various ages

	At 9 years	At 12 years	At 23 years	Endocrine dysfunction
<i>Somatotropic axis</i>				
GH peak on GH stimulation tests (μg/l)	2.4 (low)	6.4 (low)	0.9 (low)	GH deficiency
	3.8 (low)	6.7 (low)		
IGF-1 (ng/ml)	11 (–2 SDS) (low)	230 (–1 SDS) (low)	197 (–2 SDS) (low)	
<i>Thyroid hormones</i>				
Free T4 (pmol/l)	10 (low)	On L-Thyroxin	On L-Thyroxin 13.9 (normal)	Hypothyroidism
TSH (mIU/l)	4.3 (normal)		2.8 (normal)	
<i>Gonadotropic axis</i>				
LH basal/peak (IU/l)		<0.1/1.4 (low)	0.6/10.7 (low)	Hypogonadism
FSH basal/peak (IU/l)		2.7/11.6 (low)	2.1/5.9 (low)	
Oestradiol (pmol/l)		5.5 (low)	<9.9 (low)	
Ghrelin (pg/ml)			955 (high)	Hyperghrelinemia

Abbreviations: FSH, follicle-stimulating hormone; GH, growth hormone; IGF-1; insulin-like growth factor-1; LH, luteinizing hormone; SDS, SD score; TSH, Thyroid-stimulating hormone.

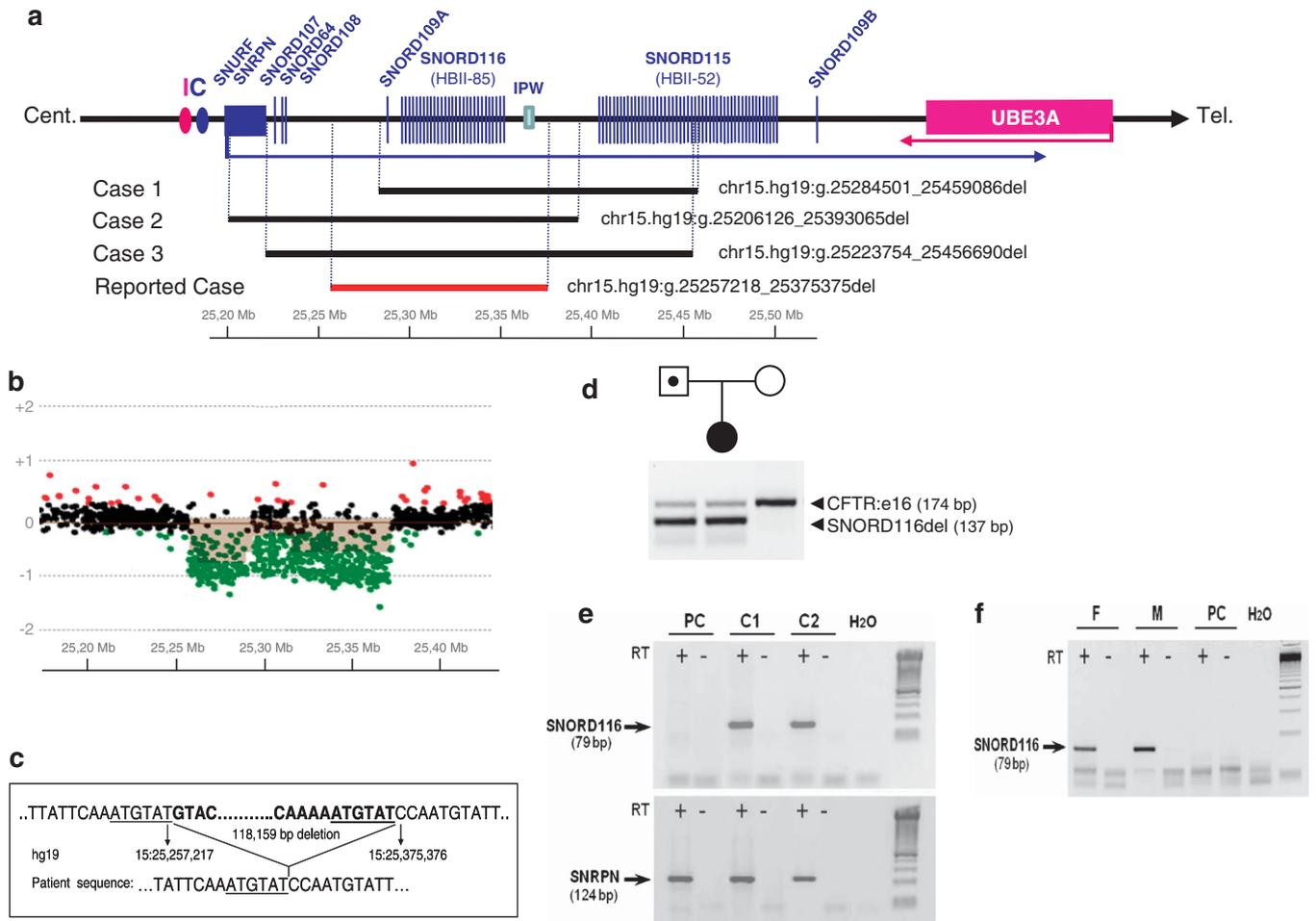


Figure 2 Characterisation of SNORD116 cluster microdeletion. **(a)** Schematic physical map of the 15q11.2 region between the SNRPN and UBE3A genes. SnoRNA genes are indicated with bars and other genes with boxes. The paternally expressed genes and the large SNURF-SNRPN transcript (arrow) are labelled in blue and the maternally expressed UBE3A gene with its transcript (arrow) in purple. The bipartite imprinting centre (IC) is indicated by two ovals. The black horizontal bars represent the three previously reported SNORD116 microdeletions (case 1,⁷ case 2,⁸ case 3⁶) and the present case with the respective genomic positions (hg19). The 15q11.2 region is represented at scale with physical distance in Mb. **(b)** High-resolution array-CGH of 15q11.2 shows loss of copy number of 333 SNP probes encompassing a segment of ≈ 116.5 kb. **(c)** Breakpoint sequence with the 118,159 bp deletion (bold) and the short direct repeat (underlined). **(d)** Confirmation of the SNORD116 deletion by a PCR assay developed after sequencing of a junction fragment obtained by PCR. The 137-bp fragment resulting in amplification of the breakpoint region is detected in the affected case and in the proband's healthy father. Amplification of exon 16 of *CFTR* gene (174 bp) is used as a PCR control. **(e)** Analysis using RT-PCR of fibroblast RNA of *SNORD116* and *SNRPN* expression. PC, present case; C1-2, normal control; **(f)** Analysis using RT-PCR of lymphocyte RNA of *SNORD116* expression. PC, present case; F, father of the present case; M, mother of the present case. RT+, with reverse transcription; RT-, without reverse transcription. Expression of the *SNORD116* cluster was negative for RT+ in lymphocytes or fibroblasts of the present case, but it was positive in his father who also carried the 118-kb deletion. On the other hand, *SNRPN* was normally expressed in the fibroblasts of the present case.

and phenotype data of the proband were deposited with the online DECIPHER database (<http://decipher.sanger.ac.uk>, patient ID: 285216).

DISCUSSION

The proband presented the complete phenotype that is characteristic of PWS, including neonatal hypotonia, endocrine phenotype with GHD, central hypothyroidism, hypogonadism and hyperghrelinemia, and cognitive and behavioural impairment.^{1,10-12} Obesity was not extreme due to the strict control of food access, but she clearly displayed hyperphagia and deficit of satiety. Overall, hyperphagia and behavioural problems are milder than generally observed in patients with PWS but typical.

We report the shortest, paternally transmitted, deletion of the *SNORD116* region that has been described to date, associated with a

clear PWS phenotype. *SNORD109A* and *IPW* were also deleted in the proband. Their role has never been highlighted in PWS pathophysiology.² Moreover, we found that *SNORD116* expression was fully abolished in lymphocytes and fibroblasts of this patient (Figures 2e and f). The father carried the deletion and because he was asymptomatic, it was strongly assumed that the deletion is located on his maternal allele. Supporting this assumption, RT-PCR analysis of his lymphocytes showed normal expression of *SNORD116* (Figure 2e). The study of the maternal parent of the father would provide the definitive answer. Unfortunately, both the parents of the father were deceased and no material was available to test this hypothesis (Supplementary Figure 3S). Comparison of demonstrative cases of PWS-like cases with short deletions of the minimal functional PWS gene locus including the *SNORD116* region⁶⁻⁸ shows that the phenotypes are very similar (Supplementary Table 1S). Nevertheless,

in the proband, *SNORD107*, *SNORD64*, *SNORD108*, *SNORD109B* and, more importantly, *SNURF-SNRPN* and the *SNORD115* cluster were not deleted (Figure 2a). In all previously described cases, at least one of these genes was preserved. Of note, deletions encompassing *SNURF-SNRPN* may result in atypical PWS phenotype.¹³ While the *SNORD115* region is genetically intact, its expression could be affected by the absence of the *SNORD116* gene cluster or hypothetical regulatory elements in the surrounding region. *SNORD115* expression in normal subjects has been shown to be present in neuronal cells and absent in peripheral tissues.¹⁴ However, de Smith *et al*⁸ using RT-PCR found an expression of *SNORD115* in the lymphocyte-derived RNA of a *SNORD116* microdeletion PWS patient while it is undetectable in his normal father. The expression of *SNORD115* (analysed by RT-PCR and microarrays) was found undetectable in the skin fibroblasts and lymphocytes of both the proband and the normal control (data not shown). These results do not rule out the possibility that the neuronal expression of *SNORD115* might be altered in our proband, but this hypothesis is impossible to verify in our living patient. Nevertheless, we can argue that the deletion of *SNORD116* gene cluster is highly contributive to generate PWS phenotype as observed in the proband. In agreement, the *SNORD116* cluster expressed in the hypothalamus seems to be regulated developmentally, playing a role in the maturation of feeding circuits.¹⁵ Knockout mice with deletion of *SNORD116* display hyperphagia.⁵ This finding is also in accordance with the recent data demonstrating the regulatory role of GC skew repeat elements or long non-coding RNA (*116HG*) derived from the *SNORD116* region.^{14,16}

This case therefore strengthens an evidence for the role of the *SNORD116* region that has recently been highlighted in the molecular pathophysiology of PWS,^{6–8} either as fully processed box C/D snoRNA species or as long non-coding host transcripts (*116HG*).^{16–19}

Next step will be to investigate the functions of these RNAs and their role in the pathogenesis of PWS. More distant genes potentially implicated in PWS syndrome as *NDN* and *MAGEL2*,² which were not deleted, were normally expressed in the fibroblasts of the proband (Supplementary Figure 4Sb). Recently, truncating mutations of *MAGEL2* were also associated with PWS.²⁰ The discussion regarding expression of *SNORD115* by non-neuronal cells can be applied to *MAGEL2*. Therefore, like for *SNORD115*, *MAGEL2* expression could not be tested here. As for *SNORD115*, it cannot be excluded that lack of *SNORD116* cluster acts as a host gene to modify *MAGEL2* expression.

Altogether, the present case suggests that the *SNORD116* is an aetiological factor in PWS. Targeted analysis of the *SNORD116* cluster by array-CGH or qPCR should be performed in patients with PW-like features in whom the diagnosis was not confirmed by more conventional cytogenetic methods and DNA methylation analysis.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Supplementary Information accompanies this paper on European Journal of Human Genetics website (<http://www.nature.com/ejhg>)