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Short report

Mosaic de novo *SNRPN* gene variant associated with Prader-Willi syndrome

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ABSTRACT

Background Prader-Willi syndrome (PWS) is an imprinting disorder caused by the absence of paternal expressed genes in the Prader-Willi critical region (PWCR) on chromosome 15q11.2-q13. Three molecular mechanisms have been known to cause PWS, including a deletion in the PWCR, uniparental disomy 15 and imprinting defects.

Results We report the first case of PWS associated with a single-nucleotide *SNRPN* variant in a 10-year-old girl presenting with clinical features consistent with PWS, including infantile hypotonia and feeding difficulty, developmental delay with cognitive impairment, excessive eating with central obesity, sleep disturbances, skin picking and related behaviour issues. Whole-exome sequencing revealed a de novo mosaic nonsense variant of the *SNRPN* gene (c.73C>T, p.R25X) in 10% of DNA isolated from buccal cells and 19% of DNA from patient-derived lymphoblast cells. DNA methylation study did not detect an abnormal methylation pattern in the *SNRPN* locus. Parental origin studies showed a paternal source of an intronic single-nucleotide polymorphism within the locus in proximity to the *SNRPN* variant.

Conclusions This is the first report that provides evidence of a de novo point mutation of paternal origin in *SNRPN* as a new disease-causing mechanism for PWS. This finding suggests that gene sequencing should be considered as part of the diagnostic workup in patients with clinical suspicion of PWS.

INTRODUCTION

Prader-Willi syndrome (PWS) is a neurodevelopmental genetic disorder that affects 1/22 000 live births in the USA.[1 2] PWS is characterised by hypotonia and feeding difficulty in infancy followed by excessive eating, obesity, global developmental delay and intellectual disability in childhood.[2 3] Absence of paternally imprinted genes in the Prader-Willi critical region (PWCR) is considered the cause of PWS. To date, three molecular mechanisms have been reported for PWS, including paternal chromosomal deletion involving the PWCR, maternal uniparental disomy (UPD) 15 and imprinting defects.[3] Paternal 15q11–q13 deletions are the most common cause of PWS. Approximately 60% of PWS result from typical paternal 15q11–q13 deletions, mostly 5–6 Mb deletions in size involving the proximal chromosome breakpoints BP1 or BP2 and the distal breakpoint BP3. About 35% of patients with PWS have maternal UPD 15, while the remaining PWS cases result from imprinting defects (microdeletions or epimutations) or chromosome translocations or inversions.[4 5] The PWCR contains a number of imprinted paternally expressed genes or transcripts. Several of these genes or transcripts have been shown to contribute to the PWS phenotype, such as *MAGEL2*, *NDN*, *SNURF-SNRPN* and *SNORD116*.[4] However, more pieces of evidence have emerged to support *SNORD116* as a major contributor to the PWS phenotypes. Angelman syndrome, the sister disorder due to imprinting error in chromosome 15q11–q13 of maternal origin, is caused by a single mutation in the *UBE3A* gene in 10%–20% of cases.[6] However, PWS has not been associated with a single point mutation of genes in the region.

In this report, we described a 10-year-old girl with a nonsense de novo mutation identified by trio whole-exome sequencing in the *SNRPN* gene with classic PWS features, such as infantile hypotonia and feeding difficulty, global developmental delay and intellectual disability, excessive eating in early childhood with central obesity, skin picking and related behaviour issues, and sleep disturbances. The consensus diagnostic criteria for PWS was assessed. To our knowledge, this is the first report to show a de novo point mutation of paternal origin within the PWCR resulting in classical phenotypes of PWS.

CASE REPORT

The patient is a 10-year-old girl who was born at 38 weeks to a 36-year-old G1P0 mother and a 49-year-old father following an uncomplicated pregnancy through caesarean section due to prolonged labour. Her birth weight was 2840 g (15%ile) and her height was 46 cm (15%ile). Her Apgar scores were 8 and 9 at 1 and 5 min, respectively. She had two dusky spell episodes during the neonatal period with an unremarkable EEG, echocardiogram and head ultrasound. She had generalised hypotonia throughout infancy with mild feeding difficulties. Developmentally, she was markedly delayed in both speech and fine motor skills. She developed PWS-related behaviours such as generalised skin picking and excessive eating noted before 5 years old (figure 1A–C). Her body weight and BMI increased to 98th percentile at the age of 6 (figure 1D). Her height has been tracking along the 50th percentile, which is normal for her mid-parental target height.

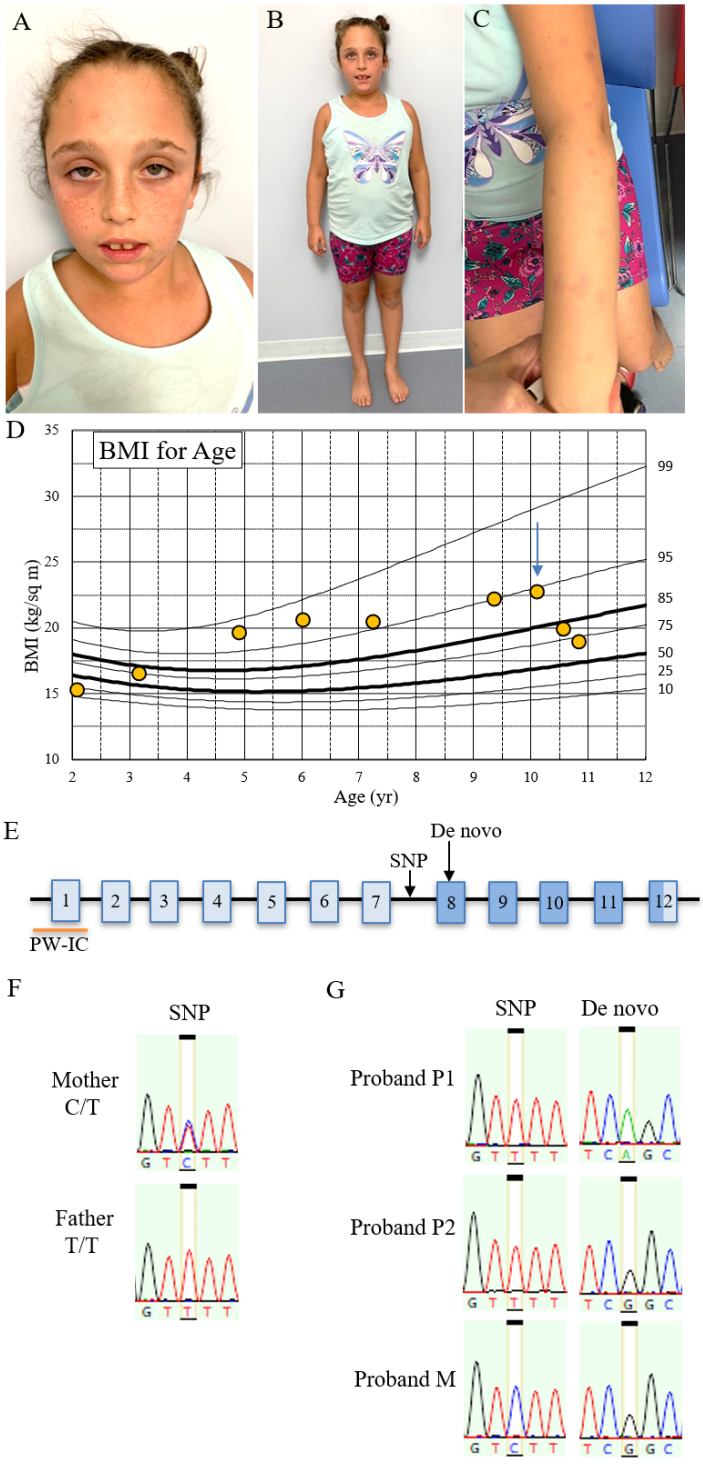

Figure 1 (A) Hypotonic facial features with mildly downslanting, almond-shaped palpebral fissures, ptosis and narrow nasal bridge. (B) Full length of the patient showing central obesity. (C) Numerous areas of skin discoloration from healed skin picking sites. (D) Growth chart for BMI with age. Arrows point to the start time for growth hormone therapy. (E) Schematic illustration of *SNRPN* gene. The location of the de novo pathogenic variant and the intronic SNP used to determine the parental origin, which is located approximately 2.6 kb upstream to the de novo variant within exon 8 of SNRPN are indicated by the arrows. The coding region is shown in dark blue. (F) DNA chromatogram showed the intronic variant C/T on the maternal specimen and T/T on paternal specimen. (G) DNA chromatogram showed the de novo variant and the SNP in the proband. Two paternal alleles of proband (P1 and P2) were shown due to mosaicism in the proband. The de novo variant was found in the paternal chromosome as evidenced by the paternal allele in proband P1. Proband M indicated the maternal allele found in the proband. The sequencing result was shown for the complementary strand in the chromatogram. BMI, Body Mass Index; PW-IC, Prader Willi imprinting centre; SNP, single-nucleotide polymorphism.

Her hands and feet were appropriate in size for age and sex. She was also diagnosed with cognitive impairment and autism spectrum disorder with attention deficit hyperactivity disorder, for which she received speech therapy, occupational therapy and applied behaviour analysis therapy. She attended special education using an Individual education plan (IEP) in the school

Table 1 Clinical features observed in proband with PWS

PWS diagnostic criteria	Proband
Neonatal hypotonia	Yes, improved with age
Feeding problems in infancy	Yes, mild
Excessive weight gain	Yes, beginning in childhood with central obesity
Facial features	Yes, almond-shaped palpebral fissures, narrow nasal bridge, hypotonic, triangular-shaped face
Hypogonadism	No
Developmental delay	Yes, global, mild
Hyperphagia	Yes, onset in childhood
Decreased fetal activity	No
Behaviour problems	Yes, autism, attention deficit hyperactivity disorder, skin picking, hyperphagia
Sleep disturbance/sleep apnoea	Yes
Short stature	No, started growth hormone therapy at age 10 years
Hypopigmentation	No
Small hands and/or feet	No
Eye abnormalities	Yes, almond-shaped palpebral fissures, bilateral ptosis
Thick, viscous saliva	No
Articulation defects	No, but has speech delay
Skin-picking	Yes, generalised

PWS, Prader-Willi syndrome.

setting. She had early signs of pubarche at 10 years of age. Her increasing Body Mass Index (BMI) was initially managed with exercise and dietary recommendations. More recently, she was started on growth hormone (GH) therapy, which greatly improved her body composition and BMI. Using the Holm *et al* consensus diagnostic criteria for PWS, the major and minor features with supporting clinical evidence represented a PWS diagnosis in this patient. The patient scored 9 points on the PWS consensus diagnostic criteria including 6 points from the major criteria.[7] Her clinical features are summarised in table 1.

Her genetic workup included a normal karyotype and Fragile X testing. Trio exome sequencing from a commercial laboratory (GeneDx, Gaithersburg, Maryland, USA) revealed a de novo mosaic nonsense variant in *SNRPN* (NM_003097.5:c.73C>T, p.R25X) in 10% of her buccal cell DNA (106× depth for this variant). To confirm the variant in another source of patient tissue, we detected this variant in 19% (772× depth) of DNA isolated from patient-derived lymphoblastoid cells (GM27891, available for distribution from the Coriell Institute for Medical Research, Camden, New Jersey, USA). This variant is very rare in the population database (gnomAD 1/251316 allele, 3.98×10^{-6} in non-Finnish European).[8] No copy number variant (CNV) was detected in the commercial laboratory using next-generation sequencing with CNV calling platform. Methylation-specific multiplex ligation-dependent probe amplification (MS-MLPA) did not detect an abnormal methylation pattern in the *SNRPN* gene using multiple probes. DNA methylation in confirming the diagnosis of PWS is greater than 99% accurate.[6 9] To determine the parental origin of the de novo variant, the trio exome sequencing data were analysed but no single-nucleotide polymorphism (SNP) could be used to identify the paternal chromosome. We then extended the SNP search to include introns within *SNRPN* by Sanger sequencing. A deep intronic SNP (NC_000015.10:g.24972855A>G, dbSNP ID rs12913403) of paternal origin approximately 2.6 kb upstream of the de novo variant was found to be useful to determine the parental origin (figure 1E). Given the low-level mosaicism, the proband's genomic DNAs were subcloned into a blunt end TOPO cloning vector (Invitrogen, Carlsbad, USA) and individual clones were selected for Sanger sequencing. The de novo variant within exon 8 of *SNRPN* was found to be present in the paternal chromosome 15 (figure 1F and G).

DISCUSSION

PWS is an imprinting disorder due to defective paternally expressed genes in the 15q11.2-q13 region. Clinically, PWS is a complex neurodevelopmental genetic disorder that typically presents with hypotonia and feeding difficulty during infancy. The feeding difficulty usually resolves with time, but hypotonia often persists. Patients with PWS classically present with multiple nutritional phases that usually resulted in hyperphagia and central obesity in late childhood.[10] Global developmental delay and moderate intellectual disability is common in patients with PWS.[2] Because of the stage-wise complex phenotype, PWS is considered a contiguous genetic disorder that result from absence of multiple paternally expressed genes due to imprinting error. Our proband has many of the classic features of PWS but relatively mild compared with most patients with PWS.

An explanation for a milder phenotype is that patients with mosaicism could have fewer symptoms and/or an incomplete phenotype as evidenced in previously reported mosaic PWS cases.[11 12] The other possibility is that the absence of the paternal SNRPN is not adequate to produce a complete PWS phenotype and severity in an affected individual. A number of cases with microdeletions involved the *SNURF-SNRPN*, and the *snoRNA* gene clusters but not the imprinting centre have demonstrated an incomplete phenotype and less severe symptoms for PWS.[13–15] These cases do suggest that the *snoRNA* cluster, especially *SNORD116*, is the major contributor to the pathogenesis of PWS as patients with microdeletions of the *SNORD116* alone do have similar phenotypes as those with both *SNURF–SNRPN* and the *snoRNA* gene clusters. Consistently, the *SNORD116* knockout mouse does recapitulate many features seen in patients with PWS.[16]

SNRPN is a small nuclear ribonucleoprotein polypeptide that is part of a large protein complex involved in pre-mRNA splicing. *SNRPN* is transcribed as the downstream gene of the bicistronic *SNURF–SNRPN* mRNA. The role of *SNURF–SNRPN* in the pathogenesis of PWS has not been fully understood. The 3′ UTR of the bicistronic *SNURF–SNRPN* gene hosts six SNORDs including *SNORD115* and *−116*.[17] It is likely that the *SNRPN* nonsense variant lead to partial degradation of the long *SNRPN* transcript that harbours the intronic snoRNA clusters *SNORD115* and *SNORD116*. A small duplication that was predicted to alter splice donor sites at the end of *SNURF/SNRPN* exon 1 was found in two affected members in a family.[18] These two patients had overgrowth, increased appetite and speech delay in childhood but not hypotonia and feeding difficulty in infancy. Several microdeletions overlapped with *SNRPN* but not the imprinting centre have been reported in the Decipher CNV database (patient IDs 331052,331554, 331186, 264659, 288263 and 293178). Some of these patients have an autistic disorder with developmental delay, but no detailed clinical information was provided. The mouse model of *SNRPN* knockout has not consistently demonstrated PWS phenotypes.[16] However, recent studies suggest SNRPN does play an important role in brain development and function.[19 20]

MAGEL2 is another imprinted gene located in the PWCR. Single-nucleotide variants in *MAGEL2* have been associated with Schaaf-Yang syndrome (SYS). Although SYS has a significant

overlap in phenotypes with PWS, they are considered as two distinct genetic disorders. given the difference in their molecular mechanisms.[3] It will be interesting to assess the impact of the nonsense *SNRPN* variant on the expression of *MAGEL2* and other imprinted genes in the PWCR in future studies.

In summary, we report a mosaic de novo nonsense variant in the *SNRPN* gene in a 10-year-old girl who presented with phenotypes that are consistent with the clinical diagnosis of PWS. To our knowledge, this is the first report to show a single nucleotide variant associated with PWS in the *SNRPN* locus. Current guidelines for PWS workup does not include gene sequencing, which may be indicated in patients with clinical suspicion of PWS but having negative DNA methylation patterns.

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