Novel Tandem Duplication in Exon 1 of the *SNURF/SNRPN* Gene in a Child with Transient Excessive Eating Behaviour and Weight Gain

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Key Words

Duplication in *SNURF/SNRPN* • Loss of *SNURF* expression • Prader-Willi syndrome

Abstract

A deletion in 15q11.2 involving the *SNURF/SNRPN* gene is the typical finding in patients with Prader-Willi syndrome. Apart from translocations disrupting this gene, no other mutation types have been described so far. We report a patient in whom a small duplication in exon 1 of the *SNURF/SNRPN* gene was diagnosed which is predicted to interrupt only *SNURF* expression. The patient was investigated due to overgrowth, increased appetite and developmental delay in childhood. This duplication was inherited from her father who carries the duplication on his paternal chromosome 15 and also had transient excessive eating behaviour as an adolescent. RNA studies showed that the duplication introduces a premature stop codon in *SNURF*.

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Prader-Willi syndrome (PWS) is a neurodevelopmental syndrome characterised by hypotonia, developmental delay and excessive appetite. The aetiology of PWS is het-

erogeneous. In about 75% of patients a paternal deletion of the region 15q11.2 can be detected. Maternal uniparental disomy of chromosome 15 is seen in 20% of patients. Three percent of patients have an imprinting defect, which in some cases results from a mutation of the imprinting centre. No alterations are detected by current methods in about 2% of patients with a clinical diagnosis of PWS [Gardner and Sutherland, 2004].

A number of patients have been reported in whom either classical or atypical PWS was due to a translocation disrupting the *SNURF/SNRPN* region [Schulze et al., 1996; Sun et al., 1996; Kuslich et al., 1999; Wirth et al., 2001] or to an atypical deletion causing partial loss of the PWS critical region in 15q11.2 [Sahoo et al., 2008; Kanber et al., 2009].

Recently, Duker et al. [2010] reported a child with infantile hypotonia, early-onset morbid obesity, and hypogonadism with a paternally inherited microdeletion at 15q11.2 involving just the SNORD116 C/D box snoRNA cluster. This case provides evidence for the importance of this region in PWS.

To the best of our knowledge, no other mutations in *SNURF/SNRPN* have been described, except for disruption by translocation or deletions. Here we report a child with speech delay and excessive appetite resulting in

Fig. 1. A Proband at the age of 2 4/12 years. Note the full face, indicative of the overweight. **B, C** Proband at the age of 11.5 years. Note the average build and tapering fingers. **A, B** The face shows hypertelorism, flat nasal bridge, bulbous nasal tip and epicanthic folds.

childhood obesity, who was found to have a paternally inherited 25-bp duplication in exon 1 of the *SNURF/SNRPN* gene.

Case Report

The proband is the second child of a non-consanguineous Caucasian couple. Her mother was 30 years old and her father was 33 years old at the time of conception. The pregnancy was uneventful with normal antenatal scans. She was delivered by normal delivery at 38 weeks with a birth weight of 2.6 kg (25th centile). She was breastfed soon after birth, and there was no history of feeding difficulty. Her motor development was normal, and she started gaining weight in late infancy. Her parents raised their first concerns about her large size, speech delay and excessive weight gain at the age of 18 months. Endocrine investigations showed normal IGF1, IGFBP3, random GH and urinary GH and the cranial MRI was normal with no pituitary abnormalities. *NSD1* sequencing was normal.

At the age of 2 years, the proband's height was 98.5 cm (+3.04 SD), the weight was 23.8 kg (+5.14 SD), and the head circumference was 51 cm (+2.13 SD). She had speech delay requiring speech therapy input. Her parents also reported behaviour abnormalities such as temper tantrums. On examination, her facial features revealed mild hypertelorism, flat nasal bridge, bulbous nasal tip and epicanthic folds (fig. 1A). The systemic examination did not reveal any abnormalities. At the age of 3 years her bone age was advanced by 1 year.

The growth parameters at the age of 11.5 years were: height 170 cm (+3.26 SD), weight 63.5 kg (+2.25 SD) and head circumference 57 cm (+2.69 SD). She had mild learning difficulties and attended a special school. Presently, she could read and write and her behaviour had improved. Her eating was controlled by her parents, and the proband was successful in controlling overeating. On examination at this follow-up review, a tall build, a long tongue, tapering fingers and mild cubita valga were noted (fig. 1B, C).

The proband's father reported that at the age of 18 years he had weighed 108 kg and had significant problems resisting food. He

described himself as having mild learning difficulties at school and dyslexia. However, as an adult he learnt how to control food intake and had a normal weight and height by his 30s.

Laboratory Methods

Cytogenetic Studies

Chromosome analysis was carried out on peripheral blood lymphocytes using standard techniques. For array CGH a 44K oligonucleotide array (Agilent) was used.

Molecular Studies

Methylation-specific PCR (MS-PCR) for *SNRPN* exon 1 was performed according to Sun et al. [1996]. Genomic DNA was bisulphited using the EZ DNA Methylation Kit (Zymo Research), followed by a single tube MS-PCR assay employing a FAM-labelled common primer (5'-CTCCAAAACAAAAACTTTAAAACCCAAATTCC-3') and 2 methylation-specific primers, one specific for the methylated allele (5'-TATTGCGGTAA-TAAGTACGTTTGCGCGGTC-3') and the other specific for the unmethylated allele (5'-GTGAGTTGGTGTAGAGTGGAGTG-TTGTTG-3'). Direct sequence analysis of *SNRPN* exon 1 was performed using the forward primer 5'-ACTGCGGCAAA-CAAGCAC-3'. The reverse strand was sequenced using primer 5'-GGCCCAAATTCGTTTATTC-3'. MS-PCR products and sequencing reactions were analysed on an ABI 3130. MS-PCR was analysed using Genemarker software and sequencing using Mutation Surveyor software.

Transcript Analysis

Total RNA from the patient and 3 voluntary controls was isolated from whole blood drawn into PAX tubes (Qiagen). The tubes were stored at room temperature for 18 h after collection to maximise the RNA yield. Total RNA extraction was performed with the related PAX Blood RNA kit (Qiagen) according to the manufacturer's instructions. cDNA was synthesised from 1 µg of RNA using the ImProm-IITM Reverse Transcription System (Promega) and PCR-amplified using the following primers: forward primer 5'-TGACGCATCTGTCTGAGGAG-3' in exon 1 and reverse

primer 5'-ACTCCAATATGGCTTTAACCA-3' spanning exons 3 and 4. The products were separated on a 2.5% agarose gel and visualised with ethidium bromide on a UV imaging system. Bands were excised from the gels, and the purified DNA fragments were sequenced.

Results

Cytogenetic Studies

Chromosome analysis revealed an apparently normal female karyotype, 46,XX. Array CGH did not show any imbalances.

Molecular Studies

Molecular testing for PWS showed that both maternal and paternal alleles were present at a normal dosage; however, the length of the paternal product was larger than expected. Sequencing of genomic DNA demonstrated that this was due to a tandem duplication of 25 bp within exon 1 of the *SNURF/SNRPN* gene (fig. 2A). The duplication includes both the ATG transcription start site utilised by the *SNURF* gene and the splice donor site at the end of exon 1.

Follow-up analysis of the parents showed that the father carried the same abnormality and that this was also present on his paternally inherited chromosome 15. The inheritance was deduced because the larger allele was the unmethylated allele (paternal). Subsequently, the duplication was shown to be absent from the proband's phenotypically normal sister, mother and paternal aunt. Grandparental samples were not available.

Transcript Analysis

The duplication results in 2 potential splice donor sites at the end of *SNURF/SNRPN* exon 1. Bioinformatic analysis using the BDGP splice site prediction program predicts that the duplication inactivates the original donor site and that instead the second donor site introduced by the duplication will be utilised. Analysis of the cDNA products revealed the presence of a larger, aberrant transcript of 289 bp in the patient compared with the 264-bp product from control cDNA. The results of the corresponding mRNA analyses are given in figure 2B. The abnormal splice product was characterised by DNA sequencing, confirming the insertion of 25 bp in the mature mRNA. This insertion causes a frame shift mutation with the introduction of a premature stop codon in *SNURF* (fig. 2C).

Discussion

We report a family with 2 members, father and daughter, both found to carry a small tandem duplication within exon 1 of the *SNURF/SNRPN* gene on the paternal chromosome 15. They both had a history of over-eating in childhood causing significant weight gain, but managed to control their eating behaviour later in life. Both had some learning difficulties, which appeared to be more severe in the daughter.

We are not aware of any other reports which describe sequence variation of just exon 1 of *SNURF/SNRPN*. The duplication seen in this family was visible on MS-PCR, which is a widely used method for the detection of PWS. Therefore, it seems unlikely that it is a common variant, as it would have been described previously. The duplication alters the reading frame of the *SNURF* transcript, which utilises exons 1–3, as the duplication introduces a premature stop codon. Since *SNURF* is normally expressed from only the paternal allele, no functional *SNURF* product will be present in carriers of the duplication. Both *SNRPN* and *SNURF* are transcribed from the same polycistronic mRNA, but transcription of *SNRPN* begins further downstream and is predicted to be unaffected by the duplication.

As the duplication is paternal in both carriers and segregates with the phenotype of over-eating, it seems possible it has a role in satiety. We acknowledge, however, that the finding in only 2 family members cannot be conclusive. Previous reports have addressed the clinical consequences of loss of *SNURF/SNRPN* expression by using phenotype-genotype correlations in patients with deletions and translocations of this gene.

Patients who had translocations disrupting only *SNURF* are very rare. We are only aware of 1 such patient, described by Sun et al. [1996], in whom a translocation disrupted *SNURF*. The patient had hypotonia, poor suck and developmental delay. He was also obese at the age of 6 months, and had an increased appetite from the age of 2 years. Length was normal for age. The translocation was of paternal origin and he was reported to have *SNRPN* expression. The patient had a different neonatal course with hypotonia and poor suck; however, the growth measurements were similar to those obtained for the patient presented in this report.

The patient described by Sahoo et al. [2008], who had a microdeletion sparing *SNURF/SNRPN*, had an almost classical PWS with over-eating, although normal height and head circumference. This is in line with the observation of de Smith et al. [2009], who reported a 19-year-old

Fig. 2. A Sequencing of genomic DNA shows tandem duplication of 25 bp within exon 1 of the *SNURF/SNRPN* gene. **B** mRNA analysis. The abnormal splice product was characterised by DNA sequencing, confirming the insertion of 25 bp in the mature mRNA. **C** This insertion causes a frameshift mutation with the introduction of a premature stop codon in *SNURF*.

patient with severe hyperphagia and obesity and a micro-deletion of only the HBII-85 snoRNA cluster. This makes it less likely that loss of *SNURF/SNRPN* is the only cause for over-eating in PWS, although it cannot be ruled out that alterations in this region contribute to a phenotype of over-eating. Furthermore loss of *SNURF* expression in the mouse is not associated with a definite phenotype [Tsai et al., 1999].

In summary therefore, there remains the possibility that a paternally inherited duplication in exon 1 of *SNURF/SNRPN*, which disrupts expression of *SNURF*, is responsible for the excessive appetite and obesity in the

patient and her father. The phenotype of over-eating and learning problems segregates with the duplication. It will take more case reports of further patients with a comparable molecular phenotype to elucidate the role *SNURF/SNRPN* plays in PWS.

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