A novel deletion of SNURF/SNRPN exon 1 in a patient with Prader-Willi-like phenotype

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ABSTRACT

Here we report the smallest deletion involving SNURF/SNRPN that causes major symptoms of Prader-Willi syndrome (PWS), including hypotonia, dysmorphic features, intellectual disability, and obesity. A female patient with the aforementioned and additional features was referred to the Mayo Clinic Cytogenetics laboratory for genetic testing. Chromosomal microarray analysis and subsequent Sanger sequencing identified a de novo 6.4 kb deletion at 15q11.2, containing exon 1 of the SNURF gene and exon 1 of the shortest isoform of the SNRPN gene. SNURF/SNRPN exon 1, which is methylated on the silent paternal allele, is associated with acetylated histones on the expressed paternal allele. This region also overlaps with the PWS-imprinting center (IC). Subsequent molecular methylation analysis was performed using methylation-specific MLPA (MS-MLPA), which characterized that the deletion of SNURF/SNRPN exon 1 was paternal in origin, consistent with the PWS-like phenotype. Since SNURF/SNRPN gene and the PWS-IC are known to regulate snoRNAs, it is likely that the PWS-like phenotype observed in patients with paternal SNURF/SNRPN deletion is due to the disrupted expression of SNORD116 snoRNAs.

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1. Introduction

Prader-Willi syndrome (PWS) is characterized by severe hypotonia and poor feeding in early infancy, followed by excessive appetite and developmental delays. Approximately 65%–75% of PWS results from deletion of the paternal copy of the imprinted genes within the PWS critical region, including SNURF/SNRPN, and the C/D box snoRNAs, such as SNORD116 (Kanber et al., 2009; Buiting, 2010). Many efforts have been made to identify the critical region responsible for PWS over several decades (Ohta et al., 1999; Tsai et al., 1999; Buiting, 2010; Cassidy et al., 2012). Previous studies have demonstrated that the exclusive deletion/duplication of SNURF/SNRPN gene, which encodes the SNURF and SNRPN polypeptides in a single transcript, is associated with classic or atypical PWS (Schulze et al., 1996; Sun et al., 1996; Conroy et al., 1997; Kuslich et al., 1999; Wirth et al., 2001; Schue et al., 2005; Buiting, 2010). For example, a patient with hypotonia, feeding problems, developmental delay, and increased appetite has been reported carrying a translocation disrupting SNURF gene (Sun et al., 1996). In 2009, another patient with similar PWS phenotype has been described with t (4; 15) (q27; q11.2) translocation with breakpoints lying between SNRPN exon 2 and 3 (Kuslich et al., 2009). In 2001, Wirth et al. reported a patient with atypical PWS phenotype carrying an apparently balanced paternally inherited t (X; 15) (q28; q12) translocation that disrupts 3′ end of the SNURF/SNRPN gene (Wirth et al., 2001). These cases suggest a critical role of SNURF/SNRPN gene in PWS.

However, recent studies have suggested that the absence of the paternally derived SNORD116 snoRNAs is a major determinant in the pathogenesis of PWS. In 2010, a patient with PWS-like phenotype, such as infantile hypotonia, early-onset morbid obesity, and hypogonadism, was reported with a paternally inherited microdeletion involving only the SNORD116 C/D box snoRNAs (Duker et al., 2010). In addition to this case, several patients with similar PWS-like clinical features have been reported carrying deletions spanning SNORD116 snoRNAs by independent research groups (Sahoo, del Gaudio et al., 2008; de Smith et al., 2009). In 2008, Sahoo et al. reported a patient with PWS carrying a 175 kb paternally derived deletion that affects several snoRNAs, including SNORD116 snoRNAs. In addition, several cases of PWS-like phenotype have been reported carrying deletions involving SNORD116 snoRNAs outside the paternally inherited microdeletion reported by Flavell et al. (2008), which spans from 15q11.2 to 0.6 Mb proximally.

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including SNORD109A, SNORD116, and SNORD115 (Sahoo, del Gaudio et al., 2008). Another patient with PWS-like phenotype, including hyperphagia, obesity and hypogonadism, was identified to carry a 187 kb deletion spanning snoRNAs (de Smith et al., 2009). Both deletions identified in these two cases overlap the SNORD116 region. Therefore, those reports have demonstrated that absence of the paternally derived SNORD116 snoRNAs plays a critical role in the PWS (Buiting, 2010).

Although PWS has been identified for several decades and many efforts have been made to understand this disease, the mechanism of PWS remains unclear. Since SNURF/SNRPN gene serves as a host for all six snoRNAs in the PWS region (Runte et al., 2001), characterizing the role of SNURF/SNRPN gene and the SNURF/SNRPN deletion is likely due to the disrupted expression of SNORD116 snoRNAs.

2. Method

2.1. Patient

Informed parental consent was obtained.

2.2. DNA extraction and Chromosomal microarray analysis (CMA)

Genomic DNA extraction and chromosomal microarray analysis were performed with the previously described methods (Cao and Aypar, 2016). CMA was performed using both copy number and single-nucleotide polymorphism (SNP) probes on a whole genome array (CytoScan HD platform) (Affymetrix). The genome-wide functional resolution of this array is approximately 30 kb for deletions and 60 kb for duplications. In addition, the array has high density coverage of the 15q11-q13 region.

2.3. Methylation-sensitive multiplex ligation-dependent probe amplification (MS-MLPA)

MS-MLPA was performed using Salsa MS-MLPA Kit ME028 (MRC-Holland). All the MS-MLPA probes used are listed in Supplemental Table 1. Briefly, 200 ng of genomic DNA was denatured at 98 °C for 10 min. Probes were then hybridized at 60 °C for 16–24 h. Upon completion, each reaction was divided into two tubes of equal volume; the ligation reaction alone was performed in one tube while ligation and simultaneous digestion with HhaI was performed in the second, both at 49 °C for 30 min. Each reaction then underwent PCR amplification using universal primers according to the manufacturer’s protocol. Upon completion, 8.5 ml Hi-Di Formamide and 0.5 ml GS-500HD Rox size standard were added to 1 ml of each reaction. This mixture was denatured at 95 °C for 5 min and placed on an ice-water slurry for an additional 5 min. Samples were then subjected to capillary electrophoresis on an ABI 3100 (Applied Biosystems). MS-MLPA results were analyzed using GeneMarker version 1.8 (SoftGenetics, LLC) in order to determine copy number and methylation status of the PWAS critical region. The fluorescent signals from the copy number probes are compared to normal controls, resulting in ratios of 0.5 for deletions and 1.5 for duplications. The methylation probes are maternally imprinted (maternal allele methylated). Therefore, when compared to normal controls, the ratio of methylated probes will increase accordingly in the presence of additional copies of the maternal allele but not due to the presence of additional paternal alleles. MS-MLPA was performed as previously described (Aypar et al., 2014).

2.4. Quantitative PCR, end point PCR, and Sanger sequencing

Ten primer sets with an expected qPCR amplicon size of 80–150 bp were designed and run on a ViiA™ 7 Real-Time PCR System to refine breakpoints of the deletion detected by microarray (Table 1). After qPCR was performed, the forward primer from the closest normal primer set proximal to the deletion (primer #2) and the reverse primer from the closest normal primer set distal to the deletion (primer #8) were used to perform end point PCR. Amplified products were verified by 0.8% E-Gel (image not shown). Sanger sequencing was then performed on the amplified PCR product to identify the exact breakpoints of the deletion (Fig. 3).

Table 1
List of the primers used for qPCR as well as CT and RQ values representing the deleted region in red (corresponding to the red boxes in Fig. 1).

<table>
<thead>
<tr>
<th>Primer Set</th>
<th>Forward Sequence (F)</th>
<th>Reverse Sequence (R)</th>
<th>Average Patient CT</th>
<th>Average Normal Control CT</th>
<th>Average RQ</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F: CTATTTCTATGCTGCTGTTGG</td>
<td>R: CTAGCAAAAAGTGAAGGTTAG</td>
<td>21.141</td>
<td>21.4</td>
<td>1.135</td>
</tr>
<tr>
<td>2</td>
<td>F: AAAAAACATTAAAAAACCGTGACA</td>
<td>R: GCTAATATGTGCTCCAGAGCG</td>
<td>23.824</td>
<td>23.91</td>
<td>1.186</td>
</tr>
<tr>
<td>3</td>
<td>F: CAACACTACCTCCTTCTATACCA</td>
<td>R: TGGCATTGGGCGGGCAGG</td>
<td>22.581</td>
<td>22.237</td>
<td>0.664</td>
</tr>
<tr>
<td>4</td>
<td>F: GCCGTTTCTACCTCTGTTGTCTG</td>
<td>R: CCTCTAACACTGCTGATTGGGA</td>
<td>24.487</td>
<td>23.267</td>
<td>0.482</td>
</tr>
<tr>
<td>5</td>
<td>F: GACACCCATGAGCCACCTC</td>
<td>R: ACTGCACTGCTTCTTCTATAA</td>
<td>23.871</td>
<td>22.925</td>
<td>0.581</td>
</tr>
<tr>
<td>6</td>
<td>F: GTCGTTCTCGGTCGCGG</td>
<td>R: ACTGCACTGCTTCTTCTATAA</td>
<td>27.864</td>
<td>26.98</td>
<td>0.61</td>
</tr>
<tr>
<td>7</td>
<td>F: AGGGGAGATAGTTATATTGGA</td>
<td>R: GGGCTCAACACCCCTATATTAA</td>
<td>24.582</td>
<td>23.552</td>
<td>0.53</td>
</tr>
<tr>
<td>8</td>
<td>F: TGGGCAGTGGGTCGCTGACATT</td>
<td>R: ATCCATCTCAGCACACACT</td>
<td>21.845</td>
<td>21.575</td>
<td>0.934</td>
</tr>
<tr>
<td>9</td>
<td>F: TGCAAGTTTCTCAGTCTG</td>
<td>R: GGGCTTCACCTGCTGCTAGA</td>
<td>22.904</td>
<td>22.87</td>
<td>1.093</td>
</tr>
<tr>
<td>10</td>
<td>F: AGCCCTTGGAGAGACCTTATG</td>
<td>R: TGGCGAATGACACACTG</td>
<td>21.844</td>
<td>21.75</td>
<td>1.048</td>
</tr>
</tbody>
</table>
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