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ARTICLE

Translocation breakpoint disrupting the host *SNHG14* gene but not coding genes or *snoRNAs* in typical Prader-Willi syndrome

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Abstract

Prader–Willi syndrome (PWS) is a well-known imprinting disorder arising from a loss of paternally imprinted gene(s) at 15q11.2–q13. We report a typical PWS patient with a balanced reciprocal translocation, 46, XY, t(15;19)(q11.2;q13.3). After Illumina whole-genome sequencing, we used BreakDancer-1.45 software to predict candidate breakpoints and manually investigated via the Integrated Genome Viewer. Breakpoint PCR followed by Sanger sequencing determined the t (15;19) breakpoints. We investigated the expression of upstream/centromeric and downstream/telomeric genes of the 15q11.2 breakpoint by reverse transcriptase PCR, using total RNA extracted from the patient's lymphoblasts. Of note, the expression of paternally expressed genes *PWAR6, SNORD109A/B, SNORD116, IPW*, and *PWAR1*, downstream of the breakpoint, was abolished. Interestingly, the breakpoint did not destroy protein coding genes or individual snoRNAs. These results indicate that these genes may play a major role in the PWS phenotype.

Introduction

Prader–Willi syndrome (PWS; OMIM 176270) is a well-known complex disorder caused by functional loss of paternally imprinted gene(s) at 15q11.2–q13. The prevalence of PWS is 1/10,000–1/30,000 [1]. PWS is characterized by neonatal hypotonia, poor feeding in infancy (often associated with failure to thrive), developmental delay, hyperphagia, and obesity in early childhood, short stature, small hands and feet, hypogonadism, and characteristic facial features. Most individuals with PWS have some cognitive impairment and behavioral problems, including temper tantrums, anxiety, and compulsive behaviors.

More than 99% of PWS patients are caused by a large deletion at 15q11.2–q13 (65–75%), maternal uniparental disomy 15 (20–30%), or imprinting defects (1–3%). Less than 0.1% of patients with PWS have a balanced translocation involving chromosome 15 [2, 3].

Studies of rare balanced translocation and small deletions have shown that *SNURF* and *SNRPN* have no major role in PWS and that *snoRNAs*, including *SNORD116* and *SNORD109*, may be functionally important for the PWS phenotype [4–6].

The human imprinted PWS gene cluster is orthologous to mouse chromosome 7C. *SNORD116/Snord116* (*PWCR1/HBII-85* in human and *Pwcr1/MBII-85* in mouse) is a gene cluster containing 30C/D box small nucleolar RNAs (snoRNAs). These snoRNAs are located in the introns of the small nucleolar RNA host gene 14 (*SNHG14*). In humans, *SNHG14* encodes a paternally expressed non-coding transcriptional unit. *SNORD116/Snord116* sequences are highly conserved in humans and mouse, and the *HBII-85* and *MBII-85 snoRNAs* are highly expressed in the

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brain [7]. In the human genome, *SNORD109A* (*HBII-438A*) is located upstream of *SNORD116*, while *SNORD109B* (*HBII-438B*) is located downstream of *SNORD115*, but there are no *SNORD109* homologs in mice [8, 9].

To date, several *SNORD116* deletion mouse models have been generated. The mice bear some characteristics reminiscent of the human PWS phenotype, including deficiencies in motor learning, memory impairment, hyperphagia, growth retardation, and increased anxiety, suggesting that *SNORD116* may be responsible for the phenotypes observed in PWS patients [7–9].

The *IPW* non-coding RNA regulates the imprinted *DLK1-DIO3* region. Some clinical features of PWS can be caused by aberrant expression of maternally expressed genes within the *DLK1-DIO3* region [10].

Here we report a clinically typical PWS patient with 46, XY,t(15;19)(q11.2;q13.3). We determined the translocation breakpoint sequences by whole-genome sequencing (WGS) and discuss a possible pathomechanism for PWS in this patient.

Materials and methods

Case report

The Ethics Committee of Hunan Jiahui Genetics Hospital approved the experimental protocols. Informed consent was obtained from the patient.

The proband is a Han-Chinese adopted boy. His birth weight was 3600 g. He had infantile hypotonia and poor feeding. He had a hyper appetite, excessive sleep, excessive weight gain, and showed obesity at 7 months of age. He started walking at 1 year, and started to speak at 1 year and 6 months. He always failed examinations at school, had poor self-control, and had frequent temper tantrums. He was hospitalized for pneumonia and heart failure at 7 years. He had a tonsillectomy for tonsil hypertrophy and surgery for otitis media at 9 years.

He visited the clinical genetics laboratory at 13 years. Physical examination showed: height 132 cm (<3rd percentile), weight 45 kg (50th percentile), head circumference 55 cm (50–75th percentile), short neck, shield chest, central obesity, small penis, and cryptorchidism. His IQ was less than 40 tested with WISC.

He was diagnosed as typical PWS by his score of 8 points satisfying with PWS based on the consensus PWS diagnostic criteria (Table 1) [6, 11–16].

Breakpoint mapping

Genomic DNA was extracted from the patient's lymphoblastoid cell line (LCL) using the QuickGene-610L DNA extraction system (Fujifilm), following the manufacturer's instructions. Non-PCR WGS was performed with 150-bp paired-end reads (Illumina). Reads were aligned to the human genome reference (hg19) using Burrows-Wheeler Aligner [17]. Base quality scores were recalibrated using GATK3.4. Candidate breakpoints were predicted using Breakdancer-1.45 [18].

Candidate breakpoints were visually inspected using the Integrative Genomic Viewer (IGV) tool [19]. The results were confirmed by breakpoint PCR and Sanger sequencing with the BigDye™ Terminator v3.1 Cycle Sequencing Kit on a 3100xl Genetic Analyzer.

Gene expression by RT-PCR

Total RNA was extracted from LCLs of the patient and four unaffected individuals using the RNeasy Plus Mini Kit (Qiagen). Total RNA was converted to cDNA using SuperScript III Reverse Transcriptase (Thermo Fischer Scientific). *SNRPN*, *PWAR5* (including *SNORD108/HBII-437*), *PWAR6*, *SNORD109/HBII-438*, *SNORD116/HBII-85, IPW, PWAR1*, and *UBE3A* RT-PCR analyses were performed. Primers were designed using Primer3 (v. 0.4.0) (http://bioinfo.ut.ee/primer3-0.4.0/primer3/) (Table S1). PCR products were separated by electrophoresis on a 1% agarose gel and were stained with ethidium bromide.

Results

Breakpoint determination

WGS successfully yielded 41.78× mean read coverage across the whole genome. Using WGS data, BreakDancer predicted 20,189 breakpoints, including 604 chromosomal translocations containing only one chr15–chr19 translocation: chr15:25237417–chr19:53424207. There was no prediction of a reciprocal chr19–chr15 translocation. Therefore, we manually searched for possible candidate breakpoints around chr15:25237417 and chr19:53424207, using the IGV (Fig. 1a, b).

We found that some parts of chr15 mapped to chr19. We then checked chr19, and found that some parts mapped to chr15. BreakDancer-1.45 did not detect any deletions around the two chromosomal breakpoints. We used flanking primers spanning possible breakpoints, and Sanger sequencing of successfully amplified products enabled us to determine nucleotide level breakpoints of der(15) and der(19) (Fig. 1c, d). No chr19 nucleotide deletions or duplications were observed at the breakpoint. However, 8 bps (ggaactta) were lost from chr15 at the breakpoint. Based on ISCN2016 nomenclature, the karyotype of this patient is 46,XY,t(15;19)(q11.2;q13.3).seq[GRCh37/hg19]t(15;19)(q11.2;q13.4) g.[chr15:

Table 1 Clinical findings in the patients with a balanced translocation

Diagnostic criteria for Prader–Willi syndrome [11]	This study	Schule et al. [6]	Schulze et al. [12]	Sun et al. [13]	Conroy et al. [14]	Wirth J et al. [15]	Kuslich et al. [16]
Major criteria (1 point)							
1. Neonatal and infantile central hypotonia with poor suck, gradually improving with age	+	+	−	+	+	−	+
2. Feeding problems in infancy and poor weight gain/ failure to thrive	+	−	+	+	−	−	+
3. Excessive or rapid weight gain after 12months but before 6 years of age; certain obesity in the absence of intervention	+	+	−	+	+	+	+
4. Characteristic facial features with dolichocephaly in infancy, narrow face or bifrontal diameter, almond-shaped eyes, small-appearing mouth with thin upper lip, down-turned corners of the mouth (3 or more required)	NA	−	+	+	NA	−	+
5. Hypogonadism: (a) Genital hypoplasia (male: scrotal hypoplasia, cryptorchidism, small penis; female: absence or severe hypoplasia of labia minora and/or clitoris); (b) delayed or incomplete gonadal maturation with delayed pubertal signs in the absence of intervention after 16 years of age	+	+	+	+	−	+	+
6. Global developmental delayed in a child younger than 6 years of age; mild to moderate mental retardation or learning problems in older children	+	−	+	+	+	+	+
7. Hyperphagia/ food foraging/ obsession with food	+	+	−	+	+	+	+
8. Deletion 15q11–13 on high resolution or other cytogenetic/ molecular abnormality of the Prader–Willi chromosome region, including maternal disomy	t(15;19)	t(4;15)	t(9;15)	t(15;19)	t(2;15)	t(X;15)	t(4;15)
Minor criteria (0.5 point)							
1. Decreased fetal movement or infantile lethargy or weak cry in infancy, improving with age	+	+	−	+	−	−	+
2. Characteristic behavior problems –temper tantrums, violent outbursts and obsessive/ compulsive behavior; tendency to be argumentative, oppositional, rigid, manipulative, possessive, and suborn; perseverating, stealing, and lying (5 or more of these symptoms required)	2/5	2/5	3/5	NA	+	2/5	3/5
3. Sleep disturbance or sleep apnea	−	+	+	−	−	−	+
4. Short stature for genetic background by age 15	+	+	+	−	−	+	−
5. Hypopigmentation—fair skin and hair compared to family	−	−	+	−	−	−	−
6. Small hands and /or feet	−	+	+	+	−	−	−
7. Narrow hands with straight ulnar border	−	−	−	−	−	−	−
8. Eye abnormalities	−	+	+	−	+	+	−
9. Thick viscous saliva with crusting at corners of the mouth	−	−	−	−	+	−	+
10. Speech articulation defects	−	−	−	−	−	−	+
11. Skin picking	−	+	+	−	+	−	+
Total score	8	8	8	9	7	6	10.5

+ present, − absent

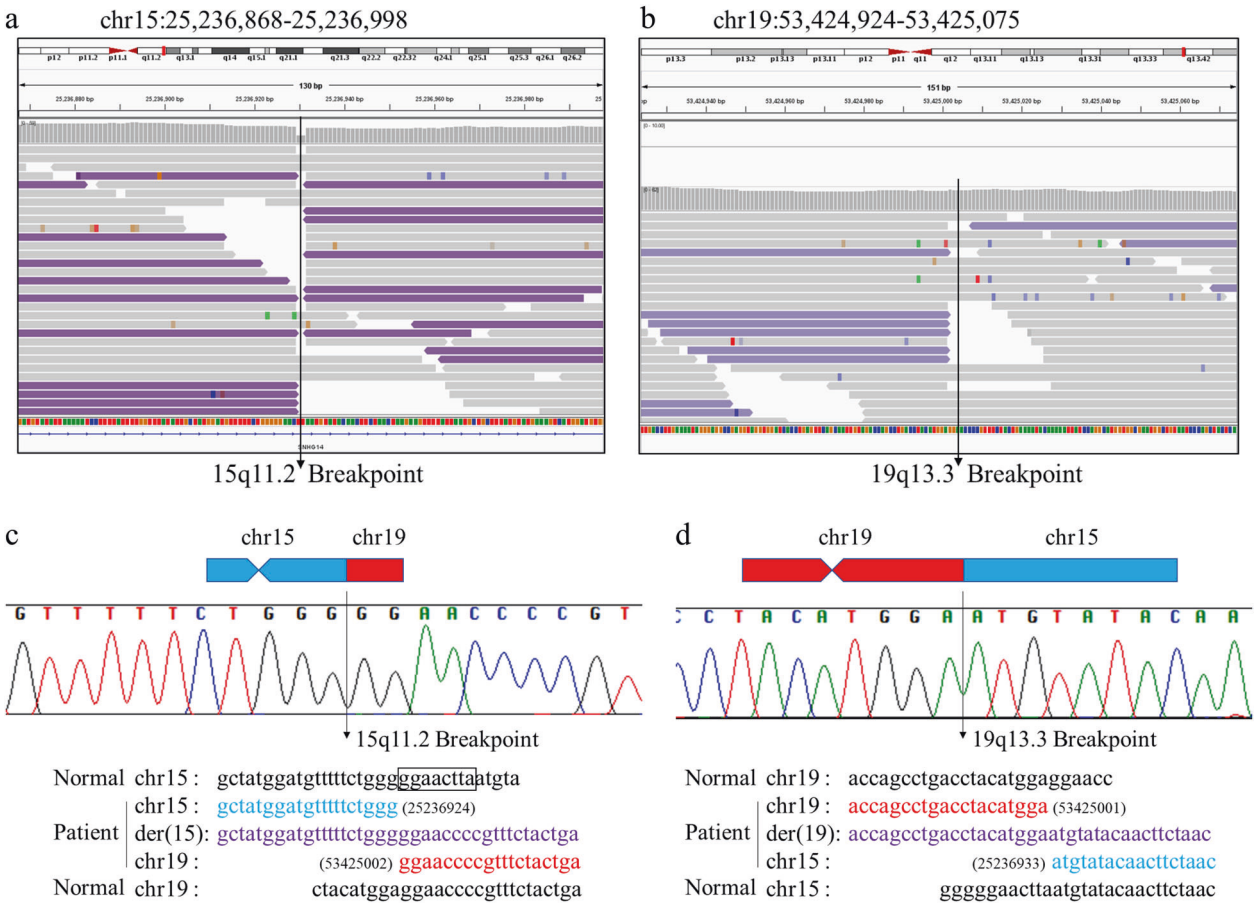

Fig. 1 Determination of translocation breakpoints. **a**, **b** Integrative Genomic Viewer results in the breakpoint regions of chr15 and chr19. **c**, **d** Breakpoint sequences of der(15) and der(19) aligned with chr15 and chr19 reference sequences

pter_cen_25236924::chr19:53425002_19qter] g.[chr19:pter_-cen_53425001::chr15:25236933_qter] [20].

Expression of upstream and downstream genes

RT-PCR of transcripts from the patient's LCLs confirmed expression of genes upstream of the breakpoint [*SNRPN* and *PWAR5* (including *SNORD108/HBII-437*)] but not of those downstream of the breakpoint [*PWAR6*, *SNORD109/HBII-438, SNORD116/HBII-85, IPW* and *PWAR1*] (Fig. 2b). Expression of the maternally expressed *UBE3A* gene was normal in the patient's LCLs, suggesting that the translocation occurred in the paternal allele (real-time PCR data not shown). All genes tested were expressed in all four controls.

Discussion

Here we report a PWS patient with a balanced reciprocal translocation, t(15;19)(q11.2;q13.3). We determined the translocation breakpoint at the nucleotide level and found that it did not disrupt any coding genes or individual *snoRNAs*, but did disrupt the host gene, *SNHG14*. The breakpoint was localized between *PWAR5* and *PWAR6*. Among previously reported patients with balanced translocations, three had breakpoints disrupting *SNRPN* (but one was not determined at the nucleotide level) [6, 12–16].

Comparison of translocation breakpoints in our patient and those previously reported revealed all breakpoints clustered within the *SNHG14* host gene. In addition, expression analysis in the LCLs of our patient and those previously reported revealed that genes downstream of breakpoints were not expressed (Fig. 2c) [6, 12, 14, 15]. These results indicate that *PWAR6*, *SNORD109A/B*, *SNORD116*, *IPW*, and *PWAR1* are functionally important and that disrupting their expression is associated with the pathogenesis of PWS in these cases. Consistent with this, the minimum critical deletion in PWS was reported to involve *SNORD109A*, *SNORD116*, and *IPW* [4, 5].

The topologically associating domains (TAD) of chr15 and chr19 were disrupted by the translocation breakpoints (Fig. 3a, b). However, gene fusion or ectopic gene expression is unlikely as the gene direction at the fusion site of TADs differs. Therefore, disruption of *SNHG14* is the most likely mechanism of PWS development in the patient presented here.

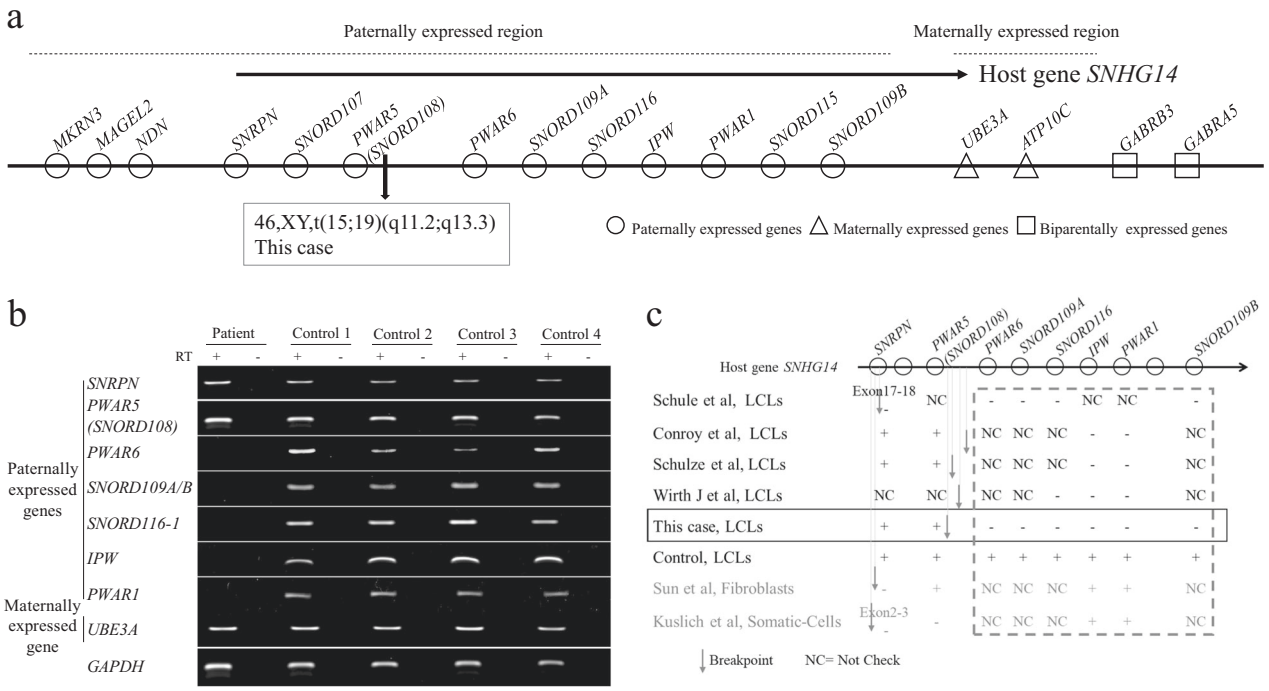

Fig. 2 Translocation breakpoint, genes, and their expression. **a** Prader–Willi syndrome region on chr15q11.2–q13. Host gene, *SNHG14*, and other individual genes are indicated as dots. **b** The RT-PCR showed that genes *SNRPN*, *PWAR5* (including *SNORD108/HBII-437*), upstream of the breakpoint, were expressed and that genes *PWAR6*, *SNORD109/HBII-438, SNORD116/HBII-85, IPW*, and *PWAR1*, downstream of the breakpoint, were not expressed. *UBE3A*, a maternally expressed gene, is normally expressed in the patient's LCLs. **c** All the patients (current and previously reported) have breakpoints in the host gene, *SNHG14*. In addition, from the LCLs gene expression analysis, downstream genes of the breakpoints were not expressed in all patients

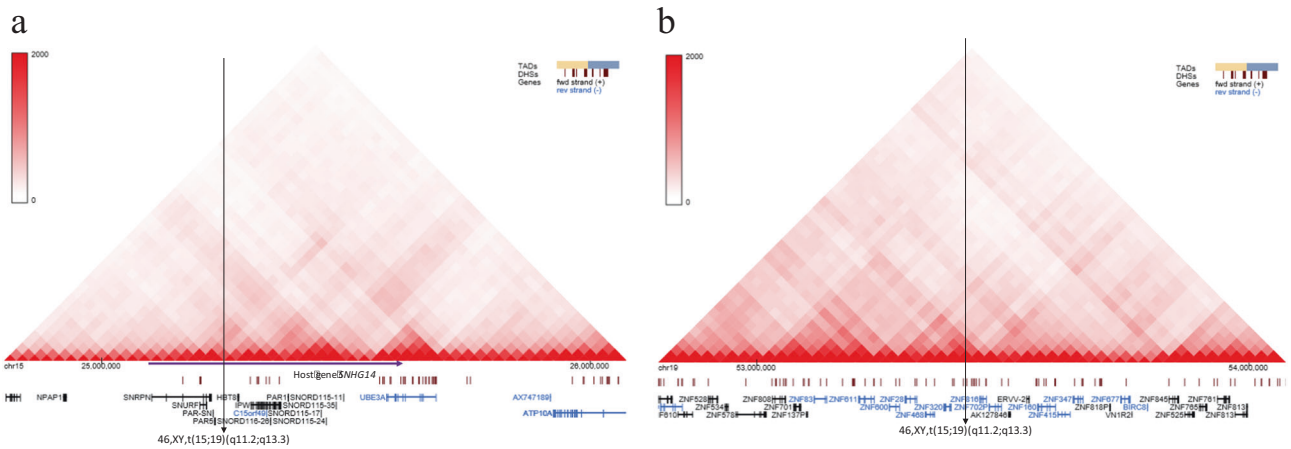

Fig. 3 Disruption of topologically associating domains (TADs) by breakpoints. **a** The TAD at chr15q11.2 was disrupted by the breakpoint. **b** The TAD at chr19 q13.3 was disrupted by the breakpoint

As this patient was adopted, we could not check the biological parents. Dysregulation of paternally expressed genes strongly suggests that the translocation occurred in the paternal chromosome. This is supported by transcription of maternally expressed *UBE3A* in the patient's LCLs.

Some *SNORD116* deletion mouse models have been generated as animal models for PWS [7–9]. The mice bear some characteristics reminiscent of human PWS phenotypes. As mice have no *SNORD109/HBII-438* homolog, there is no way to prove that genes like *SNORD109* contribute to the PWS phenotype in mice.

We identified the balanced translocation t(15;19) at the nucleotide level. This translocation disrupts the host *SNHG14* gene. Expression of genes telomeric of the breakpoint (*PWAR6*, *SNORD109A/B, SNORD116, IPW*, and *PWAR1*) should be important for the pathogenesis of PWS. These results support the minimum critical genes previously implicated as necessary for the development of PWS.

In conclusion, the translocation breakpoint in this patient suppressed transcription of *PWAR6*, *SNORD109A/B*, *SNORD116, IPW*, and *PWAR1*, which may play a major role in PWS pathogenesis. These results reiterate the importance of *SNORD109A*, *SNORD116*, and *IPW* as genes of the minimum critical deletion in PWS.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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