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Author manuscript

Hum Mol Genet. Author manuscript; available in PMC 2018 July 25.

Published in final edited form as:

Hum Mol Genet. 1996 April ; 5(4): 517–524.**Breakage in the *SNRPN* locus in a balanced 46,XY,t(15;19) Prader-Willi syndrome patient****Yongming Sun¹, Robert D. Nicholls², Merlin G. Butler³, Shinji Saitoh^{2,+}, Bryan E. Hainline^{1,4}, and Catherine G. Palmer¹**¹Department of Medical and Molecular Genetics, Indiana University Medical Center, Indianapolis, IN 46202–5251, USA²Department of Genetics, Case Western Reserve University, and Center for Human Genetics, University Hospitals of Cleveland, Cleveland, OH 44106, USA³Department of Pediatrics/Division of Medical Genetics, Vanderbilt University School of Medicine, Nashville, TN, USA⁴Department of Pediatrics, Indiana University Medical Center, Indianapolis, IN, USA**Abstract**

A patient with Prader-Willi syndrome (PWS) was found to carry a *de novo* balanced reciprocal translocation, t(15;19)(q12;q13.41), which disrupted the small nuclear ribonucleoprotein N (*SNRPN*) locus. The translocation chromosome 15 was found to be paternal in origin. Uniparental disomy and abnormal DNA methylation were ruled out. The translocation breakpoint was found to have occurred between exon 0 (second exon) and 1 (third exon) of the *SNRPN* locus outside of the SmN open reading frame (ORF), which is intact. The transcriptional activities of *ZNF127*, *IPW*, *PAR-1*, and *PAR-5* were detected with RT-PCR from fibroblasts of the patient, suggesting that these genes may not play a significant role in the PWS phenotype in this patient. Transcription from the first two exons and last seven exons of the *SNRPN* gene was also detected with RT-PCR; however, the complete mRNA (10 exons) was not detected. Thus, the PWS phenotype in the patient is likely to be the result of disruption of the *SNRPN* locus.

INTRODUCTION

Prader-Willi syndrome (PWS) is a complex genetic disorder and an example of genomic imprinting. The clinical manifestations include decreased fetal activity, neonatal hypotonia, neonatal feeding difficulties, hyperphagia with obesity, hypogonadism, short stature, small hands and feet, and mental retardation (1). About 60–70% of the individuals with PWS have been found to have an interstitial deletion at 15q11–13 (1,2). The deletion is of paternal origin (3). About 30% of PWS patients have maternal uniparental disomy of human chromosome 15 (4). The remainder have mutations in the imprinting process (5), and in a few rare cases, balanced reciprocal translocations involving chromosome 15 (1). However,

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no detailed molecular studies have been published on any of the balanced reciprocal translocation patients so far.

DNA markers specific for chromosomal region 15q11–13 have been developed and widely used for delineation of specific abnormalities in patients with PWS, including microsatellites, cosmids, and yeast artificial chromosomes (YACs) (6). Detailed physical mapping has demonstrated that the common deletion region encompasses *D15S9* (*ZNF127*) to *D15S12* (7). The smallest region of deletion overlap (SRO) for PWS has been suggested to include loci from *D15S63* (*PW71*) to *D15S174* (6,8,9). However, each of these deletions (8) affects the imprinting process over a 2 Mb region (5) (S. S. *et al*, submitted), suggesting that genes for PWS could lie anywhere in the 1.5 Mb domain from *D15S9* to *D15S174*.

It has been shown that three imprinting phenomena are found in the PWS region (10,11). Several loci in this region show parent-specific DNA methylation, including *D15S9*(*ZNF127*) (8,12), *D15S63* (*PW71*) (13), and *SNRPN* (5,14–16). DNA replication timing is reported to be asynchronous in this region (17,18). Multiple genes [*SNRPN* (14,16,19,20); *IPW* (21); *ZNF127* (M. T. C. Jong, R. D. N., in preparation)] and transcripts [*PAR-5* and *PAR-1* (15)] within 15q11–13 are expressed monoallelically from the paternal chromosome. Each of these are thus candidates to play a role in the pathogenesis of PWS, thought from genetic evidence to involve at least two paternally expressed genes (e.g., reviewed in refs 11 and 29).

Recently, the *SNRPN* gene has been mapped to the PWS critical region within the smallest deletion overlap (22). It is functionally imprinted in mouse (23,24) and human (14,16,19,20). It has been suggested that the *SNRPN* gene may be a candidate gene for PWS (14,22). Two additional upstream exons were recently found for the *SNRPN* gene (15,16), and the first exon (designated –1 or α) lies within a CpG island likely to be the promoter (5,15,16).

Here we report a patient with the PWS phenotype and a balanced reciprocal translocation t(15;19)(q12;q13.41). The translocation chromosome 15 was found to be paternal in origin. Uniparental disomy and a large deletion were ruled out, and a DNA methylation study was normal for *PW71* (*D15S63*) and *SNRPN*. Fluorescence *in situ* hybridization (FISH) with 15q11–13 probes, including subfragments of the *SNRPN* gene, together with Southern analysis with different exons from the *SNRPN* gene, established the breakpoint to be within the *SNRPN* gene. RNA expression studies by reverse transcription-polymerase chain reaction (RT-PCR) of multiple genes monoallelically expressed within 15q11–13 further implicate the *SNRPN* locus as playing a role in the PWS phenotype in this patient.

Clinical description

This 3 year 6 month old Caucasian male was referred because of obesity and behavioral problems. He was diagnosed with Prader-Willi syndrome (Fig. 1) on the basis of satisfying the consensus major diagnostic criteria (scored 8 points) and some minor diagnostic criteria (scored 2 points) for PWS (25). He was born at 42 weeks of gestation weighing 3720 g (50th centile) to a 36 year old mother following a pregnancy complicated by a 30 pound weight loss. Decreased fetal activity was also noted during the pregnancy. The child had a large

cephalohematoma at birth after a prolonged difficult delivery. He was floppy and lethargic for the first 6 months of life and had a poor suck. He weighed 10 kg (97th centile) at 6 months of age and 15 kg (>97th centile) at 18 months. His development was delayed, for example, he walked at 18 months and spoke his first words at 2 1/2 years. His appetite increased drastically after 2 years of age, and by 31 months, his weight was 24 kg (>> 97th centile). A head CT scan was normal. He had a history of hyperphagia. At 35 months of age, a Stanford Binet composite test score was at the 12th centile with below average short term memory and quantitative skills. Family history included a 5 year old sibling with cerebral palsy secondary to birth trauma and the mother had a dysplastic kidney. Physical examination at 42 months of age showed a weight of 33.8 kg (at the 50th centile for a 10 year child) with a height of 103 cm (75th centile for 42 months). He did not have hypopigmentation. His testicles were undescended. His hand length was at the 25th centile and finger length at the 10th centile. He had mild generalized hypotonia. Bone age was advanced by 9 months with a standard deviation of 6.7 months.

RESULTS

Cytogenetic analysis and parental origin of the translocation chromosome 15

Chromosome analysis with high-resolution GTG banding showed a *de novo* balanced translocation, 46, XY, t(15;19) (q12;q13.41) in all cells analyzed from peripheral blood (Fig. 2). It has been demonstrated in PWS that the syndrome is caused by the absence of paternal gene(s) in 15q11–13, most commonly either by deletion (3) or by maternal uniparental disomy (10). Chromosome polymorphism has been used for elucidation of parental origin of chromosomes (3). Therefore, the parental origin of the translocation chromosome 15 was investigated with QFQ banding of metaphase chromosomes prepared from lymphoblast cell lines of the translocation patient and his parents (Fig. 3). The intensity and size of centromere and satellite of chromosome 15 were compared in the cells of the patient with those in cells of parents, and the der(15) was established as paternal in origin.

Analysis for uniparental disomy

Uniparental disomy accounts for about 30% of PWS patients and has been found in conjunction with chromosome 15 translocations (10,26) and inv dup(15) (27). Therefore, a number of microsatellite markers were used to analyze for uniparental disomy. PCR studies of DNA loci *D15S541*, *D15S543*, *D15S63*, *D15S128*, *D15S10*, *GABRB3*, and *GABRA5* indicated heterozygosity, while *MNI*, *D15S122*, *D15S97*, *D15S219* and *D15S165* showed only one allele each, most likely indicating homozygosity for these loci. *D15S128*, *GABRB3* and *GABRA5* showed biparental inheritance (Table 1), ruling out uniparental disomy of chromosomes 15.

DNA methylation analysis

Recently, some PWS patients have been identified as having abnormal DNA methylation (imprinting mutation) of chromosome 15q11–13 (5,8,15,28,29) (S. S. *et al*, submitted) due to a microdeletion close to *SNRPN* in an element termed the imprinting center (IC) (5). Therefore, methylation analysis of loci *PW71* and *SNRPN* was performed (Fig. 4). For *PW71*, two bands (6.6 kb and 4.4 kb) are detected using the PW71B probe (30) in a normal

individual. Only one upper band (6.6 kb) is detected in a typical PWS patient. However, both bands are detected in our translocation patient (Fig. 4A). For the *SNRPN* promoter region, two bands (4.3 kb and 0.9 kb) are detected in a normal individual, only one band (0.9 kb) is detected in an Angelman syndrome patient, whereas in typical PWS patients only the upper 4.3 kb band is detected (5,16) (Fig. 4B). However, both bands were detected in our t(15;19) translocation patient. These data suggest that the t(15;19) translocation patient had a normal DNA methylation pattern and does not have an imprinting mutation.

Breakpoint determination by FISH and Southern analysis

FISH analysis was performed with cosmid probes *D15S10*, *D15S11*, *SNRPN* and *GABRB3* (Fig. 5A–D) to characterize the status of chromosome region 15q11–13. All metaphases produced hybridization signals in the cells studied from the patient. The probe *D15S11* hybridized to the der(15) chromosome, whereas *D15S10* and *GABRB3* hybridized to the der(19) chromosome. Hybridization of the *SNRPN* probe resulted in signals on both der(15) and der(19) (Fig. 5). These data indicated that no deletion of these loci had occurred and that the breakpoint was within the *SNRPN* cosmid probe. This probe contains two overlapping cosmids which span approximately 40 kb. Since the *SNRPN* gene has been proposed to be a candidate gene for PWS (14,22), it is of importance to determine the breakpoints. To identify whether the translocation breakpoint was inside the *SNRPN* gene, two approaches were used, FISH and Southern analyses.

A contig of two *SNRPN* cosmids (A and B) was obtained from Oncor (Gaithersburg, MD). Restriction analysis with *EcoRI*, *BamHI*, and *NotI* and subsequent hybridization with a cDNA probe containing *SNRPN* exons 1–8 or a cDNA probe containing exons –1, 0 and 1 was performed to deduce which fragment contained which *SNRPN* exons (Fig. 6A). When digested with *EcoRI* (Fig. 6A), cosmid A generated four fragments larger than 5 kb: 11 (F2), 8.5 (F4), 6 (F3), and 5.8 kb (F1) (Fig. 6A). Digestion of cosmid B also produced four fragments larger than 5 kb: 14 (F5), 11 (F2), 6 (F3), 5.8 kb (F6) (Fig. 6A). The subfragments were isolated from an agarose gel and labeled with digoxigenin by sequence-independent PCR. The labeled probes were hybridized to metaphase chromosomes from the patient's lymphoblasts. Fragments F1, F2 (Fig. 5E), and F5 detected signals on the der(15) while F3 (Fig. 5F), F4 and F6 detected signals on the der(19), suggesting the breakpoint is near the junction of F2 and F3 (Fig. 6A). According to the known genomic structure (Fig. 6A bottom) of *SNRPN* gene (5,15,16,22,31), the junction between fragments F2 and F3 is near exon 1 of the *SNRPN* gene. The results from this study indicate that the translocation breakpoint in chromosome 15 is within the *SNRPN* gene and located close to exon 1.

Southern blot analysis was performed by using a cDNA probe from the *SNRPN* gene exons 1–8. When genomic DNA was digested with *BamHI*, an extra novel band of approximately 20 kb was detected in the patient, but not in the parents (Fig. 6B). Southern analysis with a cDNA probe detecting exons –1, 0, and 1 uniquely was also performed. The exon 1 sequence of the probe again detected the extra 20 kb band, while the exon 0 sequence detected an abnormal extra band of approximately 17.5 kb (Fig. 6C). The exon –1 sequence of the probe detected a normal fragment of 15 kb. Abnormal fragments were also detected

with *EcoRI* and *HindIII* (data not shown). These data indicate that the breakpoint in chromosome 15 is between exon 0 and exon 1 of the *SNRPN* gene (Fig. 6A).

RNA expression analysis

Recently, several genes and transcripts in 15q11–13 have shown allele-specific expression from the paternal chromosome only. Such genes include *SNRPN* (14,16,19,20), *IPW* (21), and *ZNF127* (M. T. C. Jong, R. D. N., in preparation), and the transcripts *PAR-5* and *PAR-1* (15). These genes or transcripts are not expressed in PWS patients, while they are expressed in normal individuals, and are thus implicated in the PWS phenotype. In order to determine the expression pattern of these genes or transcripts in the translocation patient, expression of *ZNF127*, *IPW*, *PAR-1*, *PAR-5*, was performed with RT-PCR using total cellular RNA prepared from a fibroblast culture from the patient. Normal human brain mRNA and PCR in the absence of RT (RT⁻) were used as controls. Each of the latter four genes or transcripts showed expression in the control and in the t(15;19) translocation patient (Fig. 7A).

Since the translocation breakpoint on chromosome 15 was found within the *SNRPN* gene, the expression of *SNRPN* was studied. Two primers (RN175 and RN97) were used to amplify exon -1 to 8 (the entire *SNRPN* gene). RT-PCR was performed with the total RNA prepared from the fibroblast culture of the patient (Fig. 7B). While expression of *SNRPN* in normal brain was detected, expression of *SNRPN* in the translocation patient was not detected. This result is expected, since this pair of primers span the translocation breakpoint, and also shows that the maternal allele remains silent.

Although no expression of *SNRPN* was detected, the expression of exons proximal and distal to the translocation breakpoint had not been established. It is reasonable to expect that the expression of exons proximal to the translocation might still occur, since the translocation did not disrupt the promoter sequences of the gene. Therefore, the expression of proximal and distal exons was studied with RT-PCR (Fig. 7B). The expression of exon -1 to 0 was detected as expected. Surprisingly, mRNA from exons 2 to 8 was also expressed. Both products were also detected in a normal human brain mRNA control.

DISCUSSION

We have described a patient with Prader-Willi syndrome and a balanced translocation involving chromosomes 15 and 19. The patient had neither maternal uniparental disomy, nor abnormal DNA methylation. The data presented in this paper indicate that the translocation disrupted the *SNRPN* locus and is likely to be responsible for the PWS phenotype in the t(15;19) patient. Translocation PWS patients are relatively rare. The majority of PWS patients have an interstitial deletion of chromosome 15q11-q13 (1,2). In translocation patients, the majority are unbalanced involving a deletion of chromosome 15 (1,32,33). Balanced reciprocal translocation patients with PWS are described in the literature (reviewed in ref. 1), but none were studied by molecular techniques. The patient presented in this paper is unique in that no deletion is associated with the translocation. The phenotype in our patient apparently resulted from the translocation, t(15;19)(q12;q13.41), which appears to have disrupted the *SNRPN* locus.

The disruption of the *SNRPN* locus is of significance and may be responsible for the PWS phenotype. *SNRPN* was the first gene to be mapped in the PWS minimum critical region. Recent studies have indicated that the *SNRPN* gene is a strong candidate for a role in PWS (5,14–16,20,22,28). However, no mutation has been reported in the coding region of the *SNRPN* gene in PWS or PWS-like patients. Nevertheless, the expression data for other genes within 15q11–13 suggest that the PWS phenotype in our patient did not result from the lack of expression of *ZNF127*, *IPW*, *PAR-1*, or *PAR-5*, if the same expression pattern occurred in brain, the critical tissue for the neurobehavioral Prader-Willi syndrome.

The level of transcription of *SNRPN* exons, *ZNF127*, *IPW*, *PAR-1*, and *PAR-5* in fibroblast cells in the patient is not known. Whether these genes are expressed in brain cells and other tissues in the t(15;19) patient is also unknown. Thus, it is possible that an abnormal level of expression of these genes in critical tissues (e.g., brain) affects the patient's phenotype. The developmental timing of expression of these genes in critical tissues may also be important in the development of the phenotype. In addition, in balanced translocations such as this, position effects in different tissues resulting from chromatin states of the translocation partner may also determine the status of imprinted genes from 15q11–13. This could result in gene silencing in different tissues.

The translocation in the patient presented here occurred between exons 0 and 1, outside of the *SNRPN* coding region. The *SNRPN* ORF which encodes the SmN splicing factor (31,34) is still intact, and RT-PCR analysis indicates that transcription of the ORF occurs. There are three possibilities to account for the PWS phenotype in the patient: (i) the *SNRPN* ORF is in a larger protein fusion product with a putative chromosome 19 sequence, both of whose original functions are lost; (ii) the fusion mRNA is not capable of translation and no SmN function is present; and (iii) the disruption of the upstream exons of the *SNRPN* resulted in the PWS phenotype. The *SNRPN* upstream 3 exons (exons –1, 0, and 1 of the *SNRPN* locus) have been suggested to encode an additional, independent reading frame, termed *SNURF* (35) (also R. D. N. *et al*, unpublished data). Since the translocation breakpoint on chromosome 15 of the patient was found to lie between exon 0 and 1, the putative *SNURF* sequence is disrupted in this patient. Thus, the disruption of *SNURF* may play a significant role in etiology of PWS phenotype in this patient.

Based on these findings, we propose a model for the expression of *SNRPN* in this patient (Fig. 8). It has been demonstrated that *SNRPN* is expressed only from the paternal allele (14,16,19,20). The maternal allele is inactive in transcription due to imprinting (Fig. 8A). The detected expression of *SNRPN* exons 2 to 8 and exons –1 to 0, but not exons –1 to 8, indicates expression from the chromosome 15 portion in the der(15) and der(19), respectively. As was demonstrated with chromosome 15 polymorphisms, the der(15) is paternal in origin, and the chromosome 15 portion in the der(19) can also be deduced to be paternal in origin. It is reasonable to assume that the transcription level of *SNRPN* exon –1 and 0 will be roughly at the same level as if no translocation had occurred (Fig. 8B). The expression of *SNRPN* exons 2 to 8 implies that the transcription may be driven by a promoter from an unknown gene on chromosome 19 (Fig. 8C), since the translocation separated exons 1–8 from the *SNRPN* promoter and moved them to the der(19).

The expression of all paternal-only expressed loci (*SNRPN*, *ZNF127*, *IPW*, *PAR-5*, and *PAR-1*) and a normal DNA methylation pattern at loci *PW71* and the *SNRPN* promoter region suggest that paternal imprinting has been set prior to the translocation during paternal gametogenesis. It is thought that imprinting is established in the germ cells early during gametogenesis (36). Recently, it has been suggested that the translocation events in male gametogenesis most likely occur at the post-meiotic differentiating stage in spermatids (NO TAG). Therefore, it appears that the translocation in this t(15;19) patient probably occurred after imprinting was established in the paternal spermatid. Consistent with this, the loci *IPW*, *PAR-5*, and *PAR-1* downstream (telomeric) to the *SNRPN* gene and the IC (5) continue to express presumably from paternal allele, since these genes have been previously demonstrated paternal-allele expression in fibroblasts (15,21). Alternatively, it is possible that the expression of the downstream loci simply reflects the positional effect from chromosome 19. It may be that the vicinity of the translocation breakpoint in chromosome 19 is a transcriptionally open domain, which could allow *IPW*, *PAR-5*, and *PAR-1* to be transcribed in the translocation patient. In conclusion, the PWS phenotype in this patient may result from the disrupted expression of the *SNRPN* locus, rather than the lack of expression of downstream genes. Additional unique PWS patients and animal models may be necessary to definitively identify genes that play a role in the pathogenesis of Prader-Willi syndrome.

MATERIALS AND METHODS

Microsatellite analysis

PCR was undertaken for several loci from 15q11–13: *D15S541*, *D15S543*, *D15S63*, *D15S128*, *MNI*, *D15S10*, *D15S122*, *GABRB3*, *D15S97*, *GABRA5*, *D15S219* and *D15S165*. PCR amplification and polyacrylamide gel electrophoresis of ³²P end-labeled amplification products were analyzed according to modifications of the methods described by Weber and May (38). Oligonucleotide primer sequences for PCR were obtained from the NIH/DOE Genome Database (Johns Hopkins University) and from the report of the Second International Chromosome 15 Workshop (39).

FISH analysis

The hybridization and detection with probes *D15S10*, *D15S11*, *SNRPN* and *GABRB3* (Oncor, MD), was carried out according to the manufacturer's instruction. The *SNRPN* cosmid contig was provided by Oncor, Inc. (Gaithersburg, MD). *SNRPN* subfragments from *EcoRI* digestion of the *SNRPN* cosmid contig were labeled with digoxigenin by sequence-independent PCR (40). The fragments were cut out from an agarose gel, and purified with a Qiaex gel purification kit (Qiagen, CA). The purified fragments were labeled with digoxigenin by sequence-independent PCR (40). The labeled DNA was treated with DNase (200 pg/μl, Gibco BRL) for 15–20 min at room temperature. After inactivation of DNase at 65°C for 15 min, the DNA was precipitated with 100× human *Cot-1* DNA (Gibco BRL). Hybridization was carried out in hybridization solution with 50% formamide/2× SSC at 37°C for 12–18 h. Posthybridization washes were 15 min each at 42°C in 50% formamide/2× SSC and 2× SSC. Detection was performed with a detection system for

digoxigenin labeled probe (Oncor, MD). Signals were detected with a Leitz epifluorescence microscope equipped with a FITC filter and a triple-band pass filter.

Southern blot analysis

Southern blot analysis was performed according to standard methods (41). The *SNRPN* cDNA probe was synthesized by RT-PCR from total human brain mRNA. The primers were: forward 5'-GGATTTCAGGCTGAAGTGAAGG-3', reverse 5'-ACAAGA-CGCATTGCAGGGGA-3'. The PCR product was purified with the Qiaex gel purification kit, and confirmed by restriction analysis. Exon -1, 0 and 1-specific DNA probes were made by PCR from cDNA cloned in TA vectors (16).

RNA expression study

Total cellular RNA was isolated from fibroblast cultures of the patient with RNAagents™ total RNA isolation kit (Promega, WI). RT-PCR was performed essentially as described (14–16) with the exception of human normal brain mRNA. Human normal brain mRNA was a gift from Dr Lei Yu (Indiana University School of Medicine). One µg of human brain mRNA was used for reverse transcription (RT). Four to five µg of total RNA from the patient's fibroblast cultures was used for first strand cDNA synthesis with GeneAmp® RNA PCR kit (Perkin Elmer). One-tenth of the reverse transcription products were used for PCR amplification. To rule out DNA contamination, control samples contained no reverse transcriptase (RT⁻). The primers were as follows: for *SNRPN* exons -1 to 0 (16): RN175 AGAGTGGAGCGGCCGC-CGG, RN134 GCTCAGTGAGGCAGTCCTTC; exons 2–8 (16): RN84 TGGTGGAACAGCAATCATG, RN97 GATTCCA-GAACCACCTGCG; for *ZNF127*, RN153 and DD29 (Jong, M. T. C., R. D. N. *et al*, in preparation); for *PAR-1* and *PAR-5*, see Sutcliffe (15); for *IPW*, see Wevrick (21). PCR amplification was 40 cycles for 1 min each at 94°C, 60°C, and 72°C. For *PAR-1*, the PCR product was reamplified for another 30 cycles with the same conditions.

Acknowledgments

We thank Dr Lei Yu (Indiana University School of Medicine) for providing normal human brain mRNA, Susan Airhart (Oncor) for the unlabeled *SNRPN* cosmid contig, and Drs Ju Chen, Octavian Henegariu, and Nyla Heerema for suggestions and support. Funding for R. D. N. was provided by the National Institute of Health grant HD31491 and Pew Scholars Program in Biomedical Sciences.

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Figure 1. Front facial view of the proband (3 years and 6 months old). Note narrow bifrontal diameter, almond-shaped eyes, and down-turned mouth.

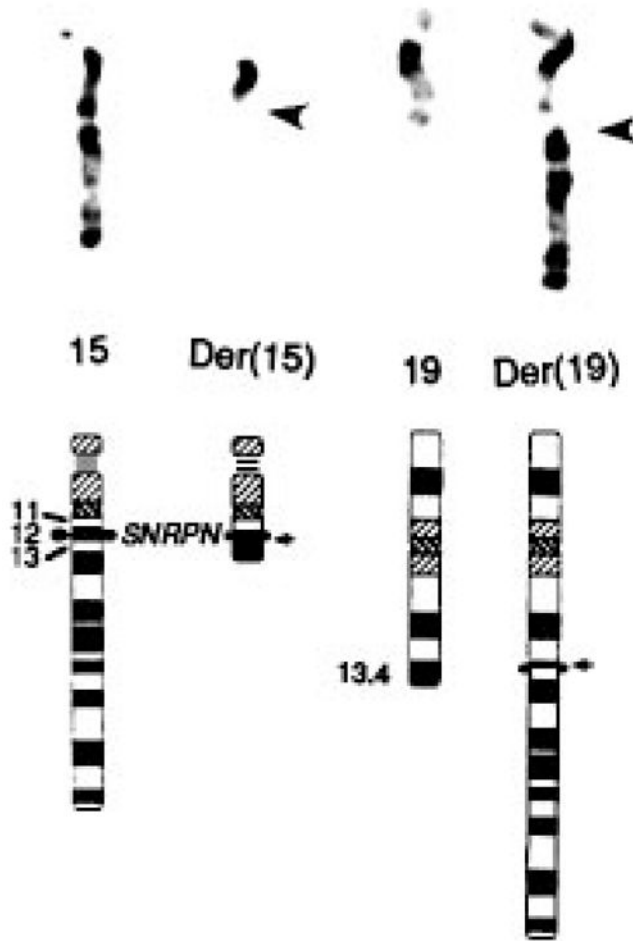


Figure 2. Chromosome GTG high resolution banding (top) showing 46,XY,t(15;19)(q12;q13.41). Only the chromosomes 15 and 19 are shown. The ideogram (bottom) shows the translocation breakpoint indicated by arrows.

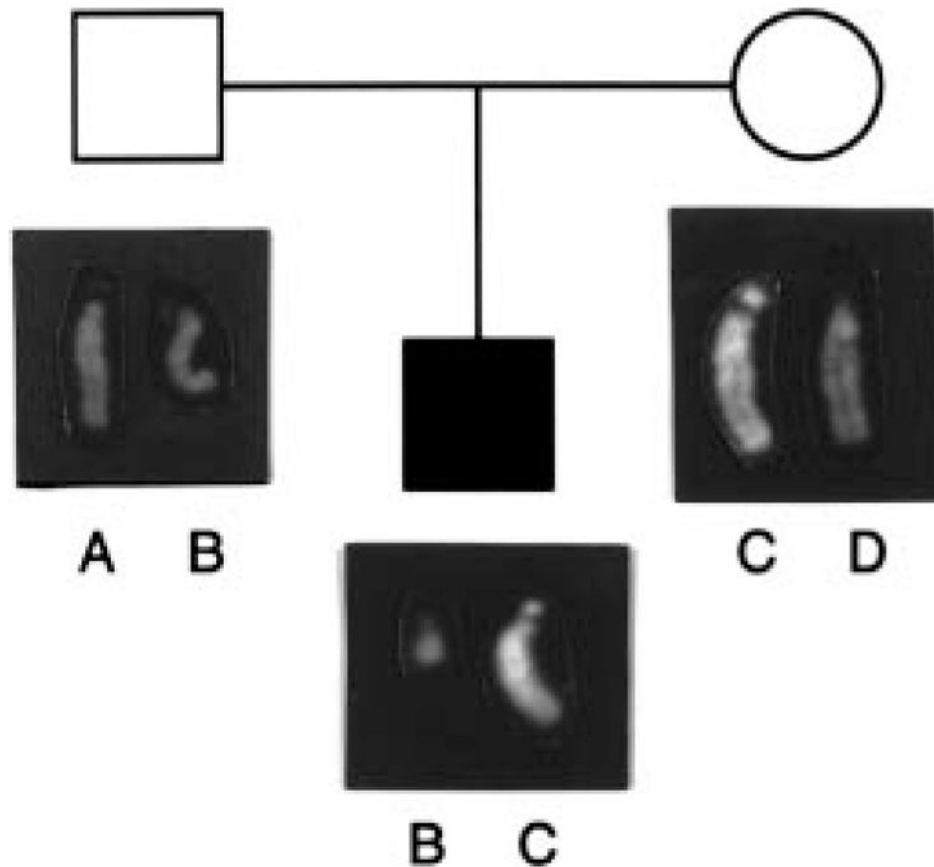


Figure 3.

Parental origin of the translocation chromosome. The normal chromosome 15 in the patient is inherited from his mother (C). The der(15) chromosome is inherited from the father (B). One of the chromosomes 15 of the father showed medium intensity centromere staining with a pale satellite. The other chromosome 15 in the father showed light centromere staining with a pale satellite. One of the mother's chromosome 15 showed a pale centromere and a bright satellite. The other chromosome 15 in the mother showed medium to pale centromere staining and a negative satellite. The patient's normal chromosome 15 showed a pale centromere and bright satellite, indicating it is maternal in origin. The der(15) showed a light centromere and pale satellite, indicating it is paternal in origin.

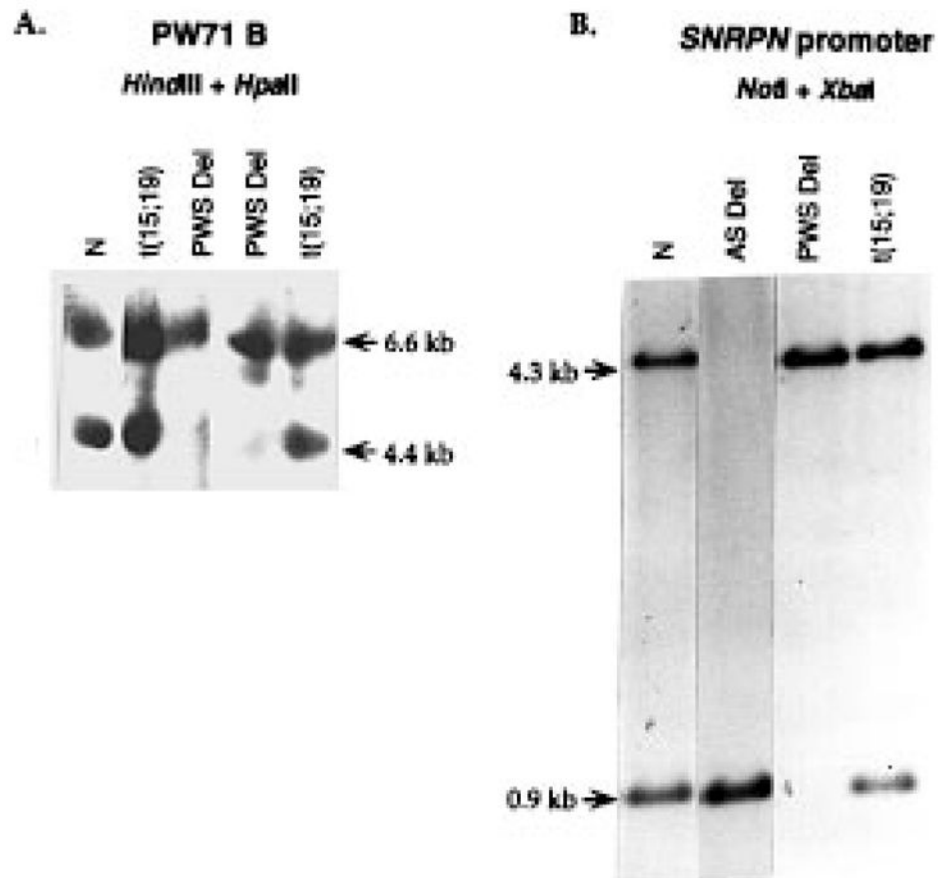


Figure 4. DNA methylation analysis. N, normal individual; t(15;19), the translocation patient described in this paper; PWS del, PWS deletion patient; AS del, Angelman deletion patient. **(A)** Leucocyte DNA was digested with *HindIII* and *HpaII* and hybridized with the PW71 B probe. The lower band (4.7 kb) is not detected in PWS deletion patients, but is present in normal individuals and the t(15;19) translocation patient. **(B)** The probe used was a cloned fragment from the *SNRPN* exon -1 region (16). DNA was digested with *NotI* and *XbaI*. The lower band is undetectable in PWS deletion patients, and for AS patients, the upper band is undetectable. Both bands are detectable in normal individuals and the t(15;19) translocation patient.

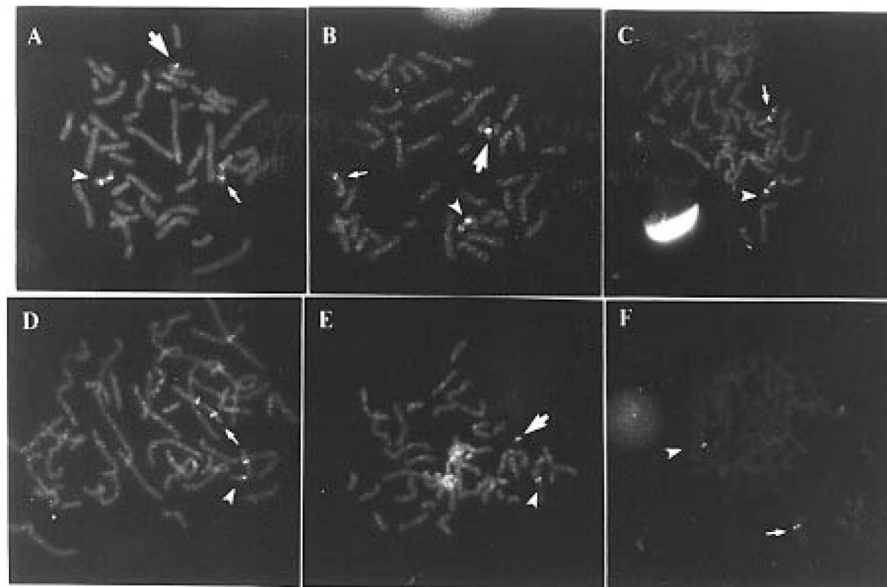


Figure 5. FISH analysis. (A) Cosmid probe *SNRPN*; (B) *D15S11*; (C) *GABRB3*; (D) *D15S10*; (E) *SNRPN* subfragment F2 (Fig. 6A); (F) *SNRPN* subfragment F3 (Fig. 6A). In A–D, the labeled probes were purchased from Oncor, and a control locus *PML* cosmid probe was also included for identification of chromosome 15. It produces signals on 15q22. Arrows indicate der(15) (large arrow), der(19) (small arrow), or normal chromosome 15 (arrow head).

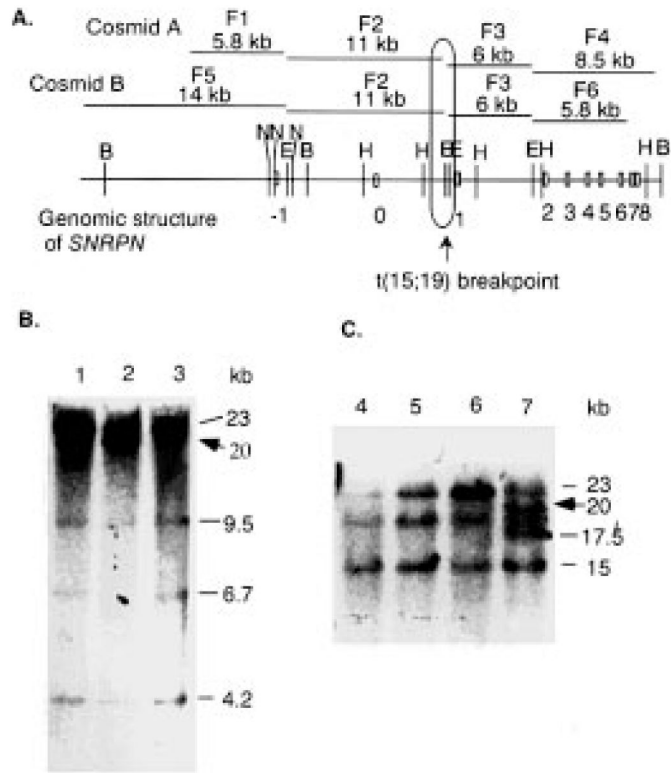


Figure 6. Identification of the translocation breakpoint. (A) Restriction map showing area around the breakpoint. Restriction analysis of *SNRPN* cosmids A and B showed the contig contained the entire *SNRPN* gene. Each cosmid produced four subfragments larger than 5 kb after digestion with *EcoRI* (F1–F6). The normal restriction map around the breakpoint is shown at the bottom (5,16). Fragment F2 (and F1, F5) hybridizes to the der(15), while F3 (and F4, F6) hybridizes to the der(19). The arrow and oblong indicate the breakpoint region, and the ten exons are indicated by numbers –1 through 8. (B, C) Southern analysis for identification of the t(15;19) translocation breakpoint. DNA was digested with *Bam*HI and hybridized with a cDNA probe (B) containing *SNRPN* exons 1–8 and a cDNA probe (C) containing exons –1, 0, and 1 (16). Lane 1, father; 2 and 7, t(15;19) proband; 3, mother; 4 and 6, PWS deletion patients; 5, PWS UPD patient; arrow, a novel fragments of 20 kb.

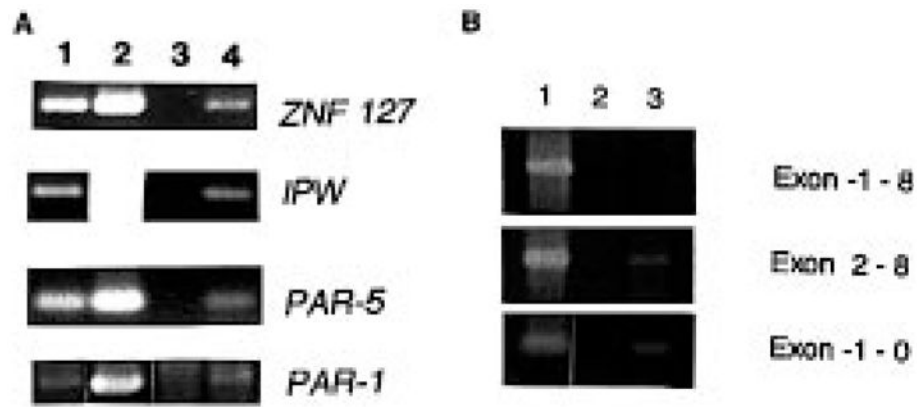
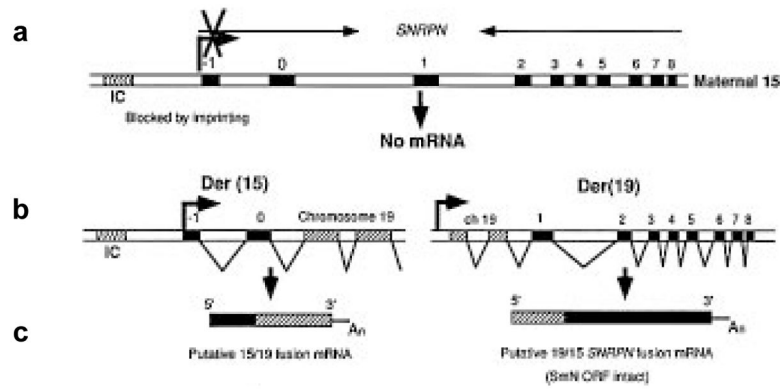


Figure 7. RNA expression analysis with RT-PCR. (A) RT-PCR analysis of *ZNF127*, *IPW*, *PAR-5*, and *PAR-1*. The amplification was present in RT⁺ reactions (lane 4), but not in RT⁻ reactions (lane 3). Lane 1, normal human brain mRNA for positive control; Lane 2, DNA derived from the translocation patient serving as positive control. It was omitted for *IPW* since RT-PCR spanned an intron. Lane 3, RNA derived from fibroblasts of the patient was incubated without reverse-transcriptase as control (RT⁻); Lane 4, RNA derived from the patient was incubated with reverse-transcriptase (RT⁺). (B) RT-PCR analysis for *SNRPN* exons proximal and distal to the translocation breakpoint. The amplification was present in RT⁺ reactions (lane 3), but not in RT⁻ reactions (lane 2). Lane 1, RT-PCR product from mRNA derived from a normal human brain; Lane 2, RT-PCR product from RNA derived from the patient's fibroblast culture without reverse-transcriptase (RT⁻); Lane 3, reaction product from RNA derived from the patient's fibroblast culture with reverse-transcriptase (RT⁺).

**Figure 8.**

Model for expression of *SNRPN* exons proximal and distal to the translocation. The maternal *SNRPN* gene is inactive because of imprinting, and therefore no mRNA from exon -1 to 8 is produced (a). The translocation breakpoint maps between exon 0 and 1, and exons 1 to 8 from chromosome 15 distal to the breakpoint were translocated to chromosome 19 near q13.41 (b, right) forming the der(19). The region of chromosome 19 distal to the chromosome 19 breakpoint was translocated to chromosome 15 forming the der(15) (b, at left). The translocation may also have disrupted a gene on chromosome 19, thus forming two fusion genes (c). The promoter for the *SNRPN* gene remains active and drives transcription of exon -1 to 0, either extending into the putative chromosome 19 gene or terminating randomly (not shown). This product was detected by RT-PCR with exon -1 and 0 primers (Fig. 8B). The promoter for the putative chromosome 19 gene is also active in fibroblasts and transcription generates a fusion mRNA with *SNRPN* exons 1–8 that can be detected by RT-PCR with primers from exon 2 and 8 (Fig. 8B). A_n, polyadenylation signal.

Table 1

Haplotypes for three informative microsatellite loci

Loci	Alleles		
	Father	Mother	t(15;19) proband
<i>D15S128</i>	1-2	2-3	1-3
<i>GABRB3</i>	3-1	1-2	3-2
<i>GABRA5</i>	1-2	2-3	1-3

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