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Balanced Translocation 46,XY,t(2;15)(q37.2;q11.2) Associated with Atypical Prader-Willi Syndrome

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Summary

The lack of normally active paternal genes in 15q11-q13, as an outcome of either a paternal deletion or maternal disomy, accounts for >95% of all patients with Prader-Willi syndrome. Other mechanisms, including imprinting mutations and unbalanced translocations involving pat 15q11-q13, have been described elsewhere. In this study, we present a patient with a rare balanced, de novo translocation—46,XY,t(2;15)(q37.2;q11.2)—involving breakage within the Prader-Willi/Angelman syndrome region of the paternal homologue, without an apparent deletion. The patient demonstrated several manifestations of the Prader-Willi syndrome but was clinically atypical. Cytogenetic and molecular studies of this case demonstrated the translocation breakpoint to be between *SNRPN* and *IPW,* with mRNA expression of *SNRPN* and *PAR-5* but absence of *IPW* and *PAR-1* expression. These results suggest that disruption of either *IPW* expression or a nearby gene by an upstream break may contribute to the Prader-Willi syndrome phenotype and that expression of *SNRPN* or other upstream genes is responsible for other aspects of the classical Prader-Willi syndrome phenotype.

Introduction

Prader-Willi syndrome (PWS) is a complex disorder characterized by neonatal hypotonia and failure to thrive, with subsequent delay of motor development. Short stature, mental deficiency, hypogonadism, short hands and feet, and hyperphagia with obesity usually manifest in early childhood (Cassidy 1984; Butler 1990). Approximately 70% of cases are caused by a loss of material in the paternal chromosome 15q11-q13 region, because of a cytogenetically visible interstitial deletion (Ledbetter et al. 1981; Butler 1990). Virtually all the remaining PWS patients are cytogenetically normal but lack paternal expression of proximal chromosome 15q, because of maternal uniparental disomy (UPD) (Mascari et al. 1992). A third class of PWS patients, with typical PWS features, involves mutations that affect the mechanism of genomic imprinting at 15q11-q13, resulting in a maternal methylation pattern on the paternally derived allele (Buiting et al. 1995; Saitoh et al. 1996, 1997). In this study, however, we report a PWS patient demonstrating a balanced de novo translocation—46,XY,t(2;15)(q37.2;q11.2)—that manifests as clinical PWS but with atypical features. Recently, two other PWS patients with apparently balanced translocations involving 15q11-q13 have been reported (Schulze et al. 1996; Sun et al. 1996). Describing the clinical phenotype of this new patient along with the molecular and cytogenetic findings may allow better correlation between a demonstrated molecular abnormality and an atypical clinical presentation of PWS.

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Patient, Material, and Methods

Clinical Report

The proband was the product of a 41-wk pregnancy of a 27-year-old gravida 4, para 3, AB 1 Caucasian mother and a Hispanic father. There were no significant complications or exposures. The fetus was normally active until 39 wk of gestation. Routine prenatal ultrasound was normal. He was delivered by cesarean section because of fetal distress, and a nuchal cord was noted. Birthweight was 3,040 g (25th–50th percentile), length was 56.0 cm (98th percentile), head circumference was 36.25 cm (50th–98th percentile), and Apgars were 8 at 1 min and 9 at 5 min. Initial examination revealed micrognathia, a 3.5-cm normal penis, normal scrotum, and cryptorchidism, the latter resolving spontaneously by age 1 wk. Endocrinological evaluation revealed the following: elevated 17-hydroxyprogesterone, 2.0 ng/ml (normal <1.5 ng/ml); Na, 135 mmol/liter; cranial ultrasound, normal; pelvic ultrasound, no uterus but presence of two ovoid structures suggestive of testes. He

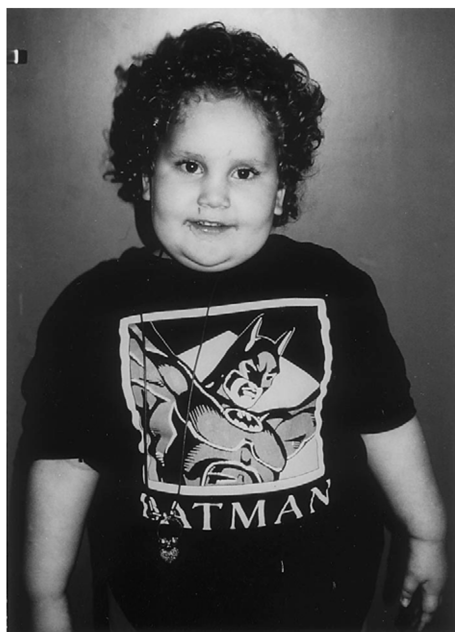

Figure 1 Proband at age 4½ years. For clinical details, see the text.

developed mild jaundice. Normal newborn genetic screening ruled out phenylketonuria, homocystinuria, maple syrup urine disease, galactosemia, hypothyroidism, and biotinidase deficiency. He had neonatal hypotonia and lethargy, with poor suck, necessitating special feeding techniques, but he never had a period of failure to thrive. He had normal amino acids, organic acids, carnitine levels, electromyogram study, and magnetic resonance–imaging scan of the brain. Very-long-chain fatty-acid analysis revealed normal C26:0 and phytanic acid; however, the ratios of C24:22 and C26:22 were slightly higher. Very-long-chain fatty acids in fibroblasts were normal.

He had alternating esotropia as an infant. His development was delayed; he sat at age 15 mo, walked at age 2 years, and first used words at age 2½ years. He had onset of obesity at age 1½–2 years, with excessive appetite and food foraging. He had behavior problems that included temper tantrums and severe aggressiveness.

He has a half-brother with poor growth and another with attention deficit/hyperactivity disorder. At age 4½ years, his weight was >95th percentile, his height was at the 50th–75th percentile, and his head circumference was >98th percentile. His hands and feet were puffy but of normal lengths (75th–97th percentile), with tapered fingers. The scrotum was normal, and penile length was at the 10th percentile. The patient had a squared nasal tip, narrow bifrontal diameter, and downturned mouth with thick viscous saliva (fig. 1). Currently, at age 5 years, he is enrolled in a special preschool setting. He exhibits a high pain threshold, has poor articulation, and is not yet toilet trained.

The proband thus has several characteristics of PWS, including neonatal hypotonia with poor suck, global developmental delay, hyperphagia, excessive weight gain, behavior problems, thick viscous saliva, and skin picking; however, he lacks several characteristic features, including small genitalia (especially, hypoplastic scrotum), a period of failure to thrive, short stature, and small hands and feet. In addition, his behavior problems began earlier and are more severe than is usual for PWS. Thus, although he satisfies diagnostic criteria for PWS, with the minimal required points, he is significantly atypical (Holm et al. 1993).

Cytogenetic Studies

Cytogenetic analysis of GTG-banded chromosomes from peripheral blood was performed on the proband and his parents, according to a modified method of Ikeuchi (1984), as we have described elsewhere (Micale et al. 1995). Chromosomes were GTG banded, and ⩾20 chromosomal spreads were examined from two cultures.

Isolation and Mapping of Phage Clones

The phage are subclones of YAC 457B4 (Buiting et al. 1995). They were mapped to the *IPW* region by pulsed-field gel analysis of the overlapping YACs 457B4 and 132D4, restriction-enzyme mapping, and hybridization with probes for *IPW, PAR-1,* and *D15S174.* Pulsed-field gel analysis and hybridization conditions were as described by Buiting et al. (1993).

FISH Studies

FISH was performed by use of both cosmids and phage probes on unstained slides, according to our previously published methods (Sullivan et al. 1996), with minor modifications. At least 10—and, in most cases, 20—metaphases were analyzed for the presence of probe signal on both the normal and the derived chromosomes.

Digital images were captured by use of a Zeiss epifluorescent microscope equipped with a cooled CCD camera (Photometrics CH250) controlled by an Apple MacIntosh computer. Gray-scale source images were captured separately with DAPI, fluorescein, and rhodamine filter sets and were merged and pseudocolored by use of Gene Join software (Yale University).

Somatic-Cell Hybrids

A lymphoblastoid cell line was established from the proband, according to the method of Neitzel (1986). Cells (5×10^{10} cells) were pelleted, washed, and subsequently mixed with a Chinese hamster cell line, Ade-c (which is deficient in GART activity), and were fused with polyethylene glycol (PEG 1500). Hybrid clones

were picked and transferred into 24-well plates. DNA was extracted from each clone and was examined by PCR with microsatellite markers from chromosome 15. After confirmation of the presence of chromosome 15 material by PCR analysis, the hybrids were analyzed by standard cytogenetic techniques, to determine whether a normal chromosome 15, a der(2) (i.e., a derived chromosome 2), or a der(15) (i.e., a derived chromosome 15) was present. Twenty-five different clones were searched until one containing the der(15) was identified.

Molecular Studies

High-molecular-weight DNA was extracted and purified from peripheral blood leukocytes, transformed lymphoblasts, and somatic cell hybrids, by use of a commercial DNA isolation kit (Puregene; Gentra Systems). Total cellular RNA was isolated from transformed lymphoblasts by use of the Purescript RNA isolation kit (Gentra Systems). Both DNA and RNA extraction were done according to manufacturers' instructions.

DNA methylation was investigated at two loci. Ten micrograms of DNA from peripheral blood and from a somatic-cell hybrid containing the der(15) was double-digested with either Not1 and Xba1 (*SNRPN* exon −1) or HindIII and HpaII (DN34/*ZNF127*), overnight at 37°C. These specimens were prepared by use of methods described elsewhere (Glenn et al. 1993; Saitoh et al. 1997). The two probes used for these methylation studies were a 1.3-kb fragment of DN34/*ZNF127* (Glenn et al. 1993) and a 650-bp PCR-amplified fragment containing exon −1 of *SNRPN* (Glenn et al. 1996). Highly polymorphic microsatellite markers for loci within 15q11-q13 (Mutirangura et al. 1993) were analyzed by PCR on peripheral blood–leukocyte DNA and on somatic-cell hybrid DNA, by standard techniques (Micale et al. 1995).

Reverse Transcriptase–PCR (RT-PCR) Studies

RT-PCR analysis of 5 μg RNA from transformed lymphoblasts from the patient, as well as controls from a PWS patient, an Angelman syndrome (AS) patient, and normal individuals, were performed for *SNRPN, PAR-5, IPW,* and the *PAR-1* transcripts, by use of standard conditions. Another 5-μg sample of RNA was used as an RT-minus control. For the primer sequences utilized for *SNRPN* (exons −1 to 8), see the reports by Glenn et al. (1996) and Sun et al. (1996); for *PAR-1* and *PAR-5,* see the report by Sutcliffe et al. (1994); for *IPW,* see the report by Wevrick et al. (1994). Products were separated on 2.5% agarose gel and were stained with ethidium bromide.

Results

Delineation of Abnormality

High-resolution cytogenetic analysis of the proband's chromosomes demonstrated an apparently balanced translocation between the distal end of the long arm of chromosome 2 and the proximal long arm of chromosome 15, with the breakpoints assigned to 2q37.2 and 15q11.2: 46,XY,t(2;15)(q37.2;q11.2) (fig. 2). FISH analysis of these chromosomes by means of a DNA probe specific for *SNRPN* hybridized to the der(15), whereas a DNA probe for *D15S10* hybridized to the der(2) (fig. 3), indicating that the breakpoint was between these two loci. FISH analysis subsequent to the initial finding, to rule out a possible deletion, was done with phages obtained from the telomeric part of YAC 457B4, which includes the *IPW* locus (Buiting et al. 1995). Phage clones λ48.14, λ48.35, λ48.48, and λ48.7 all hybridized to the der(2). However, FISH with the 12-kb phage clone λ48.34 demonstrated a split signal, with hybridization to both the der(2) and the der(15). These findings suggest that the break occurred within this 12-kb sequence and essentially exclude the presence of a deletion (figs. 4 and 5); however, a small deletion cannot be ruled out. FISH was also performed with several different YACs, to rule out the possibility of disruption of the Albright hereditary osteodystrophy (AHO) critical region on 2q (Phelan et al. 1995; Wilson et al. 1995). Hybridization with YACs 938A7 (distal to *D2S338* and *D2S345*) and 824C8 (proximal to *D2S338* and *D2S345*) demonstrated hybridization only on the der(15). Since the AHO critical region is distal to *D2S338,* these studies suggest that this region was not disrupted and was not the cause of phenotype in our patient.

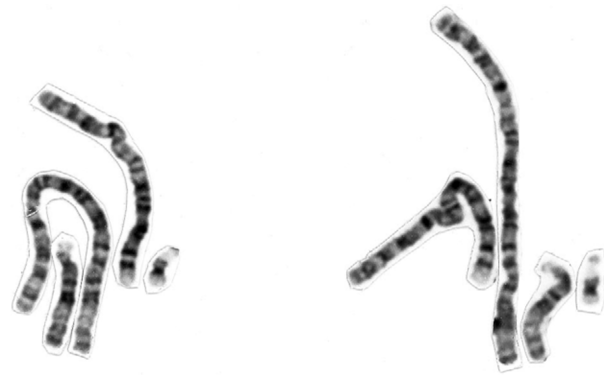

Figure 2 Partial GTG-banded karyotype of a normal chromosome 2, a normal chromosome 15, and the t(2;15)(q37.2;q11.2) translocation.

UPD Studies and Methylation Analysis

To determine whether the findings in this case were due to maternal UPD 15, microsatellite analysis of multiple sequence-tagged sites on chromosome 15 was done (table 1). Microsatellite analysis of DNA from the proband and his mother, at loci both proximal (*D15S18*) and distal (*GABRA5* and *D15S165*) to the breakpoint,

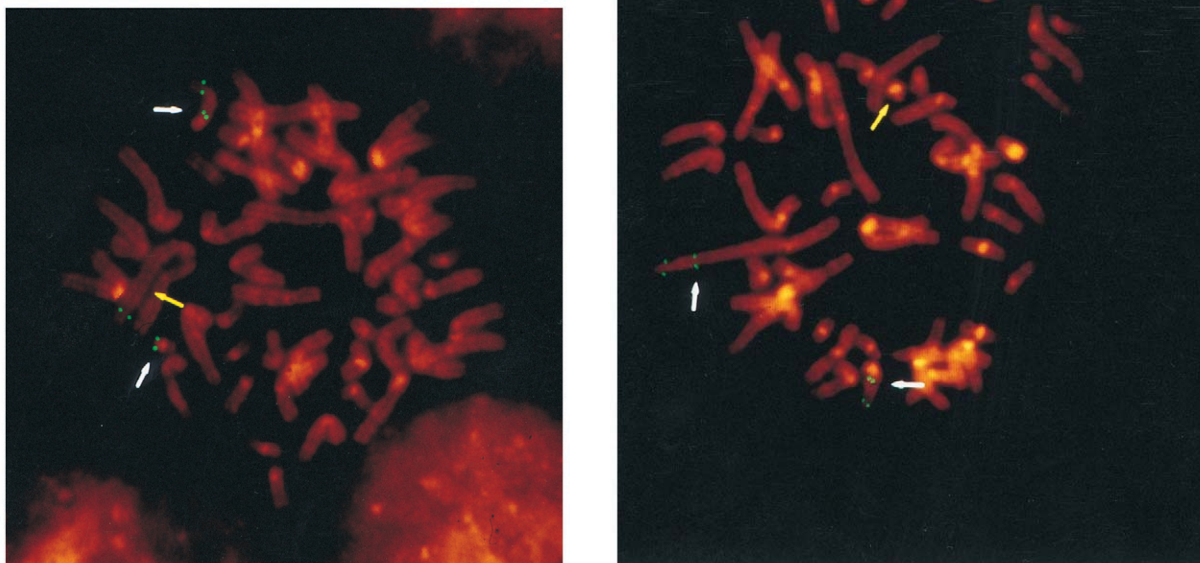

Figure 3 FISH using commercially available clone *SNRPN* and *D15S10*. The *SNRPN* probe (*left*) can be seen on both the normal chromosome 15 and the der(15) (*white arrow*) but not on the der(2) (*yellow arrow*), and the *D15S10* probe (*right*) can be seen on both the normal chromosome 15 and the der(2) (*white arrow*) but not on the der (15) (*yellow arrow*). In both photos a control probe (PML; Oncor) localized to 15q22 can be seen on both the normal chromosome 15 and the der(2).

demonstrated normal biparental inheritance. These results (table 1) exclude the presence of maternal UPD 15 as the cause of the findings in the proband.

To determine whether an imprinting mutation might be present in this proband, methylation analysis of DNA from the proband was done by use of probes for *ZNF127* (DN34) and the *SNRPN* promoter region. These studies revealed that the proband did not have an imprinting mutation.

Parental Origin of the Translocation

Cytogenetic analysis of parental blood revealed that both parents had normal chromosomes and that the rearrangement was de novo, but studies of the short arm of chromosome 15 suggested that the rearrangement was paternal in origin (data not shown). Microsatellite (table 1) and methylation analysis (data not shown) of somatic-cell hybrids revealed that the der(15) retained in the hybrid did not contain the maternal alleles, confirming the paternal origin.

RT-PCR Analysis

Recently, several different genes and expressed transcripts isolated to the PWS/AS critical region have shown parent-specific products. The genes for *SNRPN* (Glenn et al. 1993, 1996; Nakao et al. 1994; Reed and Leff 1994), *IPW* (Wevrick et al. 1994), as well as the expressed transcripts for *PAR-1* and *PAR-5* (Sutcliffe et al. 1994), yield paternal-specific products only. RT-PCR studies revealed a product for *SNRPN* and *PAR-5* in the patient, whereas for the *IPW* gene and the *PAR-1* expressed transcript, both located below the break, no product could be detected (fig. 6).

Discussion

Although the majority of cases of PWS are associated with either a visible deletion of proximal 15q (Ledbetter et al. 1981; Butler 1990) or maternal UPD (Robinson et al. 1991; Mascari et al. 1992), more-recent studies have also implicated a defect in the putative imprinting center on chromosome 15q (Sutcliffe et al. 1994). Despite this understanding, the genes and pathogenesis responsible for this common genetic disorder remain elusive. In this study we have reported an individual with a balanced translocation involving the PWS/AS critical region of chromosome 15 who exhibits a PWS-atypical phenotype. This case, together with two previously pub-

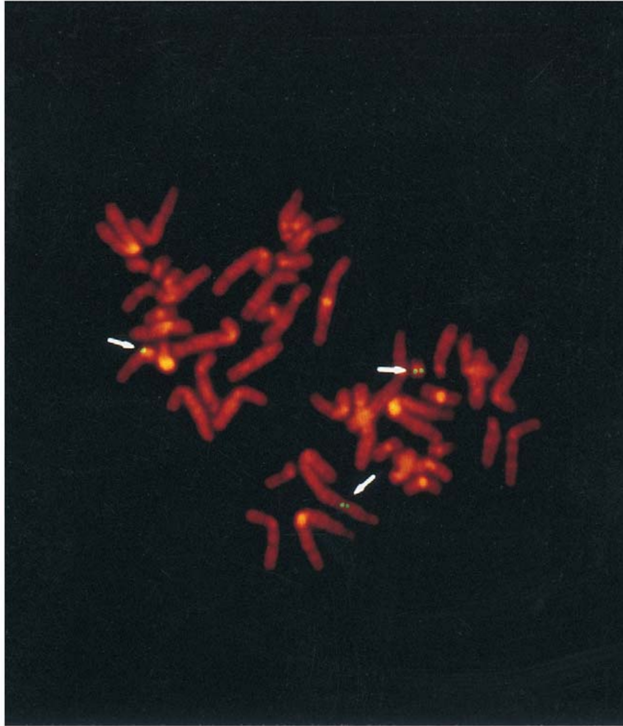

Figure 4 FISH using phage clone λ48.34. FISH with this probe not only showed hybridization to the normal chromosome 15 but also demonstrated a split signal hybridizing to both the der(2) and the der(15).

lished cases, has the potential to contribute significantly to our understanding of which genes within the deleted region may contribute to the phenotype.

Delineation of the Abnormality

High-resolution chromosomal analysis of the proband in this study revealed an apparently balanced translocation that involves a break in 15q11-q13. Subsequent FISH with phage probes revealed one phage clone (λ48.34) that was split between the der(2) and the der(15) in the proband. Since this probe was only 12 kb in size, it has been assumed that no deletion is present,

Table 1

Microsatellite Analysis of Mother, Proband, and Hybrid Containing der(15)

Locus	Mother	Proband	Hybrid[a]
D15S541	1,1	1,2	2
D15S18	2,2	1,2	ND
D15S11	1,2	2,2	ND
D15S128	2,2	1,2	1
D15S122	1,3	2,3	. . .
D15S113	1,2	1,1	ND
GABRA5	2,2	1,2	. . .
D15S111	1,1	1,1	. . .
D15S165	1,3	1,2	ND

[a] ND = not determined; an ellipsis (. . .) denotes absence.

but this can be confirmed only by cloning and sequencing the breakpoint DNA on both the der(2) and the der(15). Studies with somatic-cell hybrids demonstrated that the de novo rearrangement was paternal in origin. However, methylation analysis of two loci, which could indicate an abnormality in the paternal imprinting, was normal. Therefore, no explanation for the proband's phenotype could be derived from this information.

Since no deletion or methylation defect could be detected, RT-PCR was used to study two paternally imprinted genes (*SNRPN* and *IPW*) and two paternally imprinted transcripts (*PAR-1* and *PAR-5*) from the PWS/AS critical region (Sutcliffe et al. 1994; Wevrick et al. 1994; Glenn et al. 1996). Expression of *SNRPN* and *PAR-5,* both of which are located 5′ to the breakpoint, was detected in lymphoblast cells. However, no product could be detected for either *IPW* or *PAR-1,* both of which are located 3′ to the break. Three possible explanations for the lack of *IPW/PAR-1* expression are suggested. First, proper imprinting may not have occurred, and hence the rearrangement occurred prior to resetting of the imprint. Second, some of the PWS genes are silenced because of another mechanism (such as deletion of gene promotors/enhancers or position effects). Third,

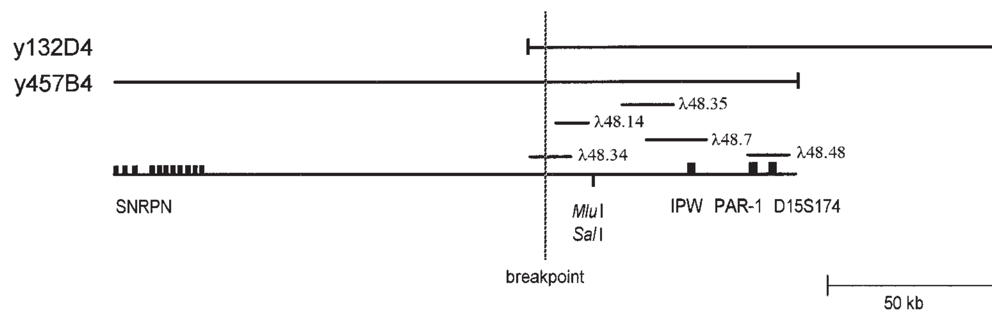

Figure 5 Physical map of the breakpoint region. Phage clone λ48.34 spans the breakpoint in the patient.

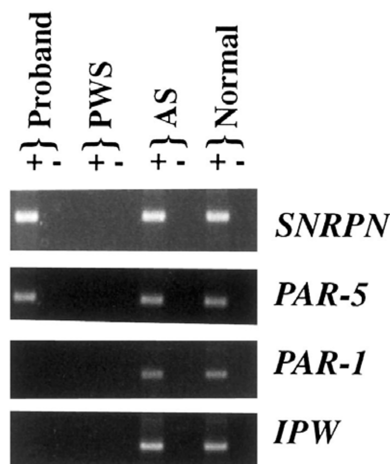

Figure 6 RT-PCR analysis showing the products of *SNRPN, PAR-5, IPW,* and *PAR-1* in the normal controls and in an AS patient. The PWS deletion patient does not show a product in either the genes or the transcripts. The proband shows a product for *SNRPN* and *PAR-5* but not for *IPW* or *PAR-1*.

the pattern of gene expression may be subject to position effect (Bedell et al. 1996; Milot et al. 1996).

Correlation With Other Reported Balanced Translocations

Including this case, there have now been three reported cases of either typical or atypical PWS associated with a balanced translocation. These studies are likely to provide considerable insight into the understanding of the pathogenesis of PWS. Although our proband has several features of PWS, he lacks others (Holm et al. 1993) and appears to have been significantly atypical; specifically, he does not have small hands and feet, hypogonadism, or the failure to thrive, which usually are associated with this syndrome. Additionally, he is at the 75th percentile for height and at the 98th percentile for head circumference. In the case reported by Schulze et al. (1996), the break was also localized between *SNRPN* and *IPW*. As in our case, they could find expression only of *SNRPN* and *PAR-5,* not of *IPW* or *PAR-1*. The patient reported by Schulze et al. also is not short (5′6″ [169 cm]), has an atypical fat distribution, and has normal hands. In contrast to these two cases, Sun et al. (1996) presented a case with classical PWS in which the break was within the *SNRPN* locus; *SNRPN* (exons −1 to 8) was not expressed, but *IPW* (along with other transcripts) was expressed.

The analysis of the three balanced-translocation cases would suggest that *SNRPN* is probably a major—but not the only—determinant of a PWS phenotype. The presence of expression of *SNRPN* with the lack of expression of either *IPW* or an adjacent gene can lead to a proband with an "atypical" form of PWS, indicating the importance of *IPW* and/or other genes in contributing to classical PWS manifestations.

The study by Sun et al. (1996) would suggest that the imprint most likely was set prior to the translocation event. Alternatively, genes may be activated by position effect, or, less likely, it may be possible for the signal from the imprinting center to function in a *trans* configuration. In addition, the precise location of the break might be of importance. It is possible that, both in the case that we studied and in that reported by Schulze et al. (1996), an uncharacterized portion of the *IPW* gene (e.g., the promoter region) may be broken, leading to the lack of expression. Characterization of the *IPW* gene promoter and identification of molecular signatures of imprint setting (e.g., DNA methylation) telomeric to *SNRPN* will allow further understanding of how balanced translocations in and around the *SNRPN-IPW* genomic region cause PWS and PWS-like phenotypes.

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