

Report

Disruption of the Bipartite Imprinting Center in a Family with Angelman Syndrome

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Imprinting in 15q11-q13 is controlled by a bipartite imprinting center (IC), which maps to the *SNURF-SNRPN* locus. Deletions of the exon 1 region impair the establishment or maintenance of the paternal imprint and can cause Prader-Willi syndrome (PWS). Deletions of a region 35 kb upstream of exon 1 impair maternal imprinting and can cause Angelman syndrome (AS). So far, in all affected sibs with an imprinting defect, an inherited IC deletion was identified. We report on two sibs with AS who do not have an IC deletion but instead have a 1–1.5 Mb inversion separating the two IC elements. The inversion is transmitted silently through the male germline but impairs maternal imprinting after transmission through the female germline. Our findings suggest that the close proximity and/or the correct orientation of the two IC elements are/is necessary for the establishment of a maternal imprint.

Prader-Willi syndrome (PWS [MIM 176270]) and Angelman syndrome (AS [MIM 105830]) are caused by the loss of function of imprinted genes in proximal 15q. In ~2%–4% of patients, this loss is due to an imprinting defect. These patients have apparently normal chromosomes 15 of biparental origin, but in PWS the paternal chromosome carries a maternal imprint and in AS the maternal chromosome carries a paternal imprint. In 15%–20% of cases, the incorrect imprint is caused by a microdeletion of the imprinting center (IC) (Sutcliffe et al. 1994; Buiting et al. 1995; Saitoh et al. 1996; Ohta et al. 1999a, 1999b; Buiting et al. 2000). The IC has been mapped to the *SNURF-SNRPN* locus and appears to have a bipartite structure. In families with PWS, the smallest region of deletion overlap (PWS-SRO) is 4.3 kb and includes exon 1 (Ohta et al. 1999a). These deletions appear to prevent the establishment or maintenance of the paternal imprint. In families with AS, an 880-bp region 35 kb upstream of the exon 1 represents the smallest region of deletion overlap (AS-SRO; Buiting et

al. 1999a). The AS-SRO contains one of several alternative upstream exons of *SNURF-SNRPN* (u5, Färber et al. 1999). We have proposed that the AS-SRO interacts with the PWS-SRO to establish the maternal imprint (Dittrich et al. 1996) and that transcripts using the alternative exons may play a role in this interaction. Transgenic experiments reported by Shemer et al. (2000) suggest that the AS-SRO does indeed interact with the PWS-SRO. If the multicopy transgene, which consists of the human AS-SRO and the mouse minimal *Snurf-Snrpn* promoter, reflects the endogenous situation, this interaction involves an element close to exon u5 rather than an alternative upstream transcript. We have now obtained tentative evidence for an interaction between the two IC elements at the endogenous human locus.

The siblings presented when the younger one (AW) was seen, with developmental delay, at age 1 year. Her older brother (DW), then age 19 years, was mentally handicapped. Three other siblings were unaffected. A diagnosis of AS was made on the basis of the pattern of learning problems and severe language impairment (neither sib has developed recognizable speech). AW is now 10 years old and has a characteristic behavioral phenotype. Ataxia is minimal, and epilepsy is absent, although suggestive EEG features have been noted. Both sibs have head circumferences above the 50th percentile, eating disorders, and obesity. These findings are similar

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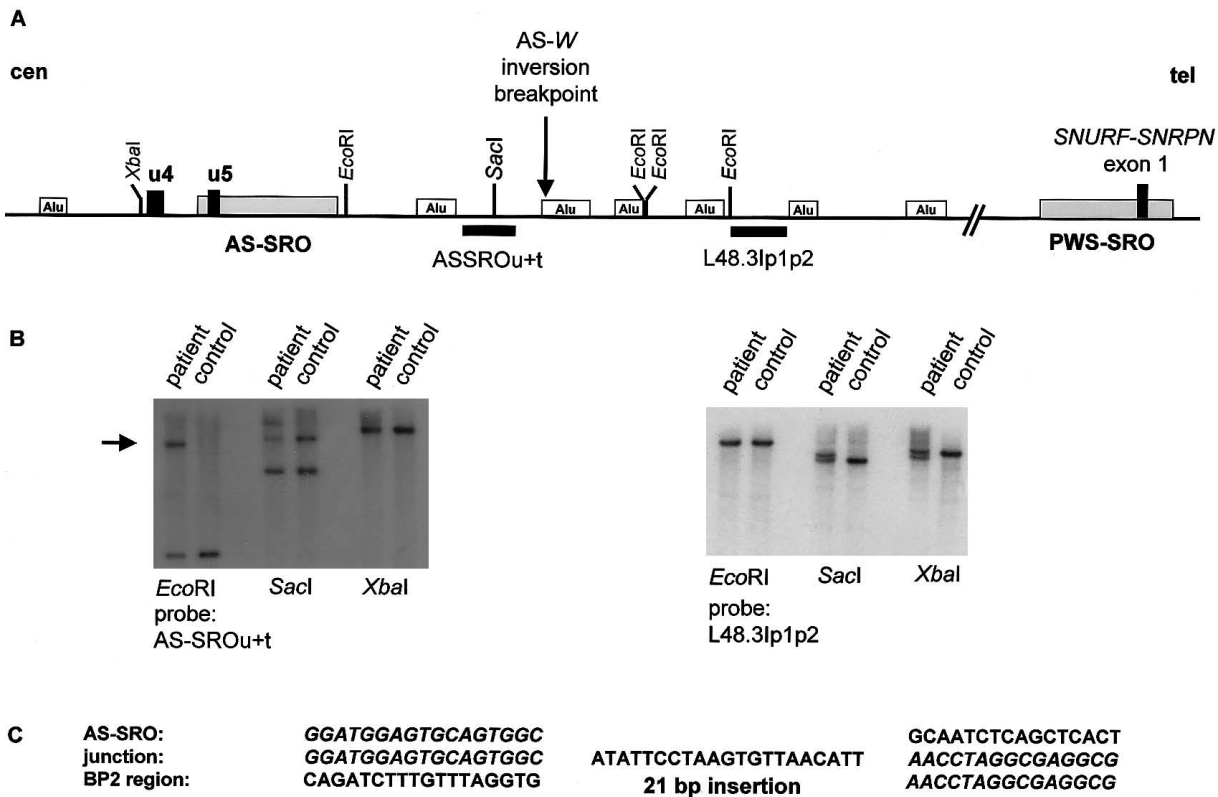


Figure 1 A, IC region. The AS-SRO and the PWS-SRO are indicated by gray boxes. Blackened boxes indicate the *SNRPN* upstream exons u4 and u5. The inversion breakpoint inside the IC region is indicated by an arrow. B, Southern blot analysis of *EcoRI*-, *SacI*-, and *XbaI*-digested DNA from patient AW and from a normal control with probe AS-SROu+t (left) and probe L48.3Ip1p2 (right). The abnormal *EcoRI* fragment, detected with probe AS-SROu+t, which was subcloned, is indicated by an arrow. C, Partial sequences of the inversion breakpoints. The breakpoint junction fragment contains a 21-bp insertion of unknown origin.

to those in the patients with AS reported by Gillissen-Kaesbach et al. (1999).

Methylation analysis of *MKRN3*, *D15S63*, and *SNURF-SNRPN* in a brother and sister with AS revealed a typical pattern—the complete loss of the maternal band. The other family members had a normal pattern (data not shown). A common large deletion and uniparental disomy was excluded by microsatellite analysis, since both affected children showed biparental inheritance of *D15S9*, *D15S11*, and *GABRB3*. Thus, the patients were classified as having an imprinting defect. All five sib pairs with AS and an imprinting defect studied to date had an inherited IC deletion (Buiting et al. 1998). Detailed Southern blot analysis using probes for the AS-SRO and flanking regions did not reveal any evidence for such a deletion. However, with a PCR-derived probe (AS-SROu+t), which maps 1 kb distal to the AS-SRO, we obtained abnormal restriction fragments in *EcoRI*- and *SacI*-digested DNA. Using another probe (L48.3Ip1p2), which maps 1 kb more distal, we also found abnormal

fragments in *SacI*- and *XbaI*-digested DNA, but these fragments differed from those detected with the AS-SROu+t probe (fig. 1B). These results suggested the presence of a structural rearrangement. By subcloning and partial sequence analysis of one of the abnormal fragments (7.1-kb *EcoRI*), we obtained evidence for an inversion with one breakpoint in an *Alu* repeat mapping 1.6 kb distal to the AS-SRO.

To localize the second inversion breakpoint, we hybridized the junction fragment to 16 overlapping YAC clones covering the entire chromosomal region 15q11-q13. We found two positive YAC clones (y962D11 and y931C4), which map inside the common deletion breakpoint cluster regions of 15q11-q13 (data not shown). These breakpoint cluster regions represent duplicated sequence stretches with a high sequence similarity and a size of ~400 kb. There are two duplicated copies—BP1 and BP2—in the centromeric region and two—BP3A and BP3B—in the telomeric region (Christian et al. 1999) (fig. 2). Since YAC clone y931C4 had been linked to the

centromeric BP2 region and YAC y962D11 to the telomeric BP3 region (Buiting et al. 1999b; Ji et al. 1999), we performed refined Southern blot analysis using 11 YAC clones, which had previously been linked to the different breakpoint cluster regions. Our results suggested that the second inversion breakpoint mapped either inside BP2 or BP3A. We then compared the sequence of PCR products covering 2.5 kb from BP2 and BP3A YACs with the sequence of the junction fragment (table 1). Although the sequences show a high degree of similarity (>99.5%), we found single-nucleotide differences at 13 positions. Seven of these nucleotide differences could be identified as single-nucleotide polymorphisms (SNPs), but at six positions the differences were specific for the two BP regions. On the basis of the diagnostic differences, we assigned the second inversion breakpoint to BP2 (table 1). The inversion spans ~1–1.5 Mbp and disrupts the IC: the AS-SRO has been removed from the PWS-SRO in the center of the imprinted domain close to its proximal border and is in an inverted orientation (fig. 2).

By Southern blot hybridization and an inversion-specific PCR assay (fig. 3A), we demonstrated that the grandfather, his twin brother, the mother, and the patients have the inversion. Since the mother inherited the inversion from her father and is healthy, the inversion is without deleterious effect when transmitted through

Table 1**Specific Nucleotide Differences between BP2 and BP3A Sequences**

	NUCLEOTIDE AT					
	nt1	nt2	nt3	nt4	nt5	nt6
Junction fragment	G	A	C	A	A	G
y931C4/BP2	N ^a	A	C	A	A	G
y409C4/BP2	G	A	C	A	A	G
y943D8/BP3A/B	T	T	T	G	G	A
y963B2/BP3A	T	T	T	G	G	A

^a The sequence of y931C4 was ambiguous. The diagnostic nucleotide “G” for BP2 was verified by sequencing two additional YAC clones derived from this locus.

the male germline. The paternally expressed genes on the inverted segment are probably not affected in the mother, as judged by methylation and expression studies of *MKRN3* (fig. 3B). This suggests that this region can be paternally imprinted irrespective of its orientation. When the inversion was transmitted through the female germline, it prevented maternal imprinting of the whole domain and led to AS. The data suggest that the close proximity and/or the correct orientation of the AS-SRO and the PWS-SRO are necessary to establish a maternal imprint, although we cannot exclude the possibility that it is only the relative position of the AS-SRO within the imprinted domain that matters.

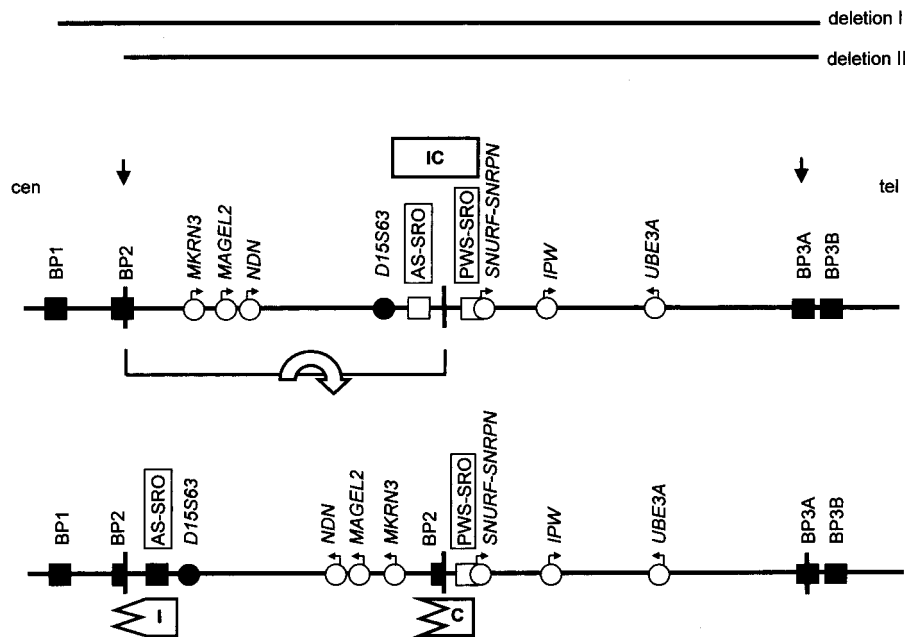


Figure 2 Chromosomal region 15q11-q13. The extent of the common large deletions in patients with PWS and with AS are shown at the top of the figure. In class I deletions, the proximal breakpoint lies inside BP1. In class II deletions, the breakpoint lies inside BP2. The normal gene order in 15q11-q13 is given in the middle of the figure. The inversion is shown at the bottom.

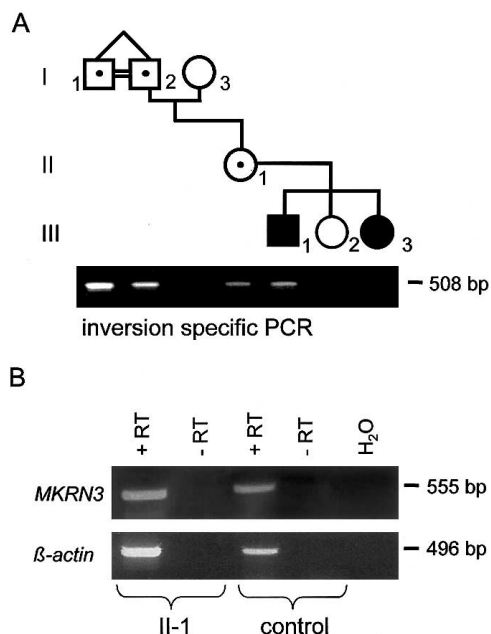


Figure 3 A, Inversion-specific PCR in family AS-W. A 508-bp PCR product, specific for the inversion, was observed in the grandfather, his twin sib, the mother, and both affected sibs. Primer sequences for this PCR are: 5' CAGCAT-GTAGCATGTATCTTTCTCA 3' (Weju3) and 5' CAGTATCCATTAGGG-ATTTGCAG3' (Weju4). B, Expression analysis. DNase I-treated RNA, extracted from lymphoblastoid cells of the mother (II-1), was positive for *MKRN3*, as was RNA from a normal control. The integrity of the RNA samples was shown by amplification of a 496-bp transcript fragment from the β -actin locus. The RT-PCR primers are as follows: DD29 and RN153 for *MKRN3* (Jong et al. 1999) and for β -actin, β -actin-F, and β -actin-R (F = 5'-TTGCTAT-CCAGGCTGTGCTATCCC-3', R = 5'-AGCACTGTGTTGGCGTAC-AG-3'). +RT = RT-PCR with reverse transcriptase; -RT = RT-PCR without reverse transcriptase; H₂O = RT-PCR without RNA.

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Electronic-Database Information

Accession numbers and URL for data in this article are as follows:

Online Mendelian Inheritance in Man (OMIM), <http://www.ncbi.nlm.nih.gov/Omim> (for PWS [MIM 176270] and AS [MIM 105830])

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