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Sporadic Imprinting Defects in Prader-Willi Syndrome and Angelman Syndrome: Implications for Imprint-Switch Models, Genetic Counseling, and Prenatal Diagnosis

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Summary

The Prader-Willi syndrome (PWS) and the Angelman syndrome (AS) are caused by the loss of function of imprinted genes in proximal 15q. In ~2%–4% of patients, this loss of function is due to an imprinting defect. In some cases, the imprinting defect is the result of a parental imprint-switch failure caused by a microdeletion of the imprinting center (IC). Here we describe the molecular analysis of 13 PWS patients and 17 AS patients who have an imprinting defect but no IC deletion. Heteroduplex and partial sequence analysis did not reveal any point mutations of the known IC elements, either. Interestingly, all of these patients represent sporadic cases, and some share the paternal (PWS) or the maternal (AS) 15q11-q13 haplotype with an unaffected sib. In each of five PWS patients informative for the grandparental origin of the incorrectly imprinted chromosome region and four cases described elsewhere, the maternally imprinted paternal chromosome region was inherited from the paternal grandmother. This suggests that the grandmaternal imprint was not erased in the father's germ line. In seven informative AS patients reported here and in three previously reported patients, the paternally imprinted maternal chromosome region was inherited from either the maternal grandfather or the maternal grandmother. The latter finding is not compatible with an imprint-switch failure, but it suggests that a paternal imprint developed either in the maternal germ line or postzygotically. We conclude (1) that the incorrect imprint in non–IC-deletion cases is the result of a spontaneous prezygotic or postzygotic error, (2) that these cases have a low recurrence risk, and (3) that the paternal imprint may be the default imprint.

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Introduction

The Prader-Willi syndrome (PWS, MIM 176270) and the Angelman syndrome (AS, MIM 105830) are distinct neurogenetic disorders that are caused by the loss of function of oppositely imprinted genes in proximal 15q (for a review, see Lalande 1996). The *UBE3A* gene has been found to be mutated in several AS patients (Kishino et al. 1997; Matsuura et al. 1997) and is expressed from the maternal allele only, at least in the brain (Albrecht

et al. 1997; Rougeulle et al. 1997; Vu and Hoffman 1997). The gene or genes for PWS have not yet been identified, but there are a number of candidate genes that are expressed from the paternal allele only (listed in order from centromere to telomere): *ZNF127* (R. D. Nicholls, unpublished data), *NDN* (Jay et al. 1997; MacDonald and Wevrick 1997), *SNRPN* (Özcelik et al. 1992; Glenn et al. 1993; Nakao et al. 1994; Reed and Leff 1994), and *IPW* (Wevrick et al. 1994). It is likely that PWS results from the loss of function of at least two of these or additional genes.

Most patients with AS or PWS have a 3–4-Mb deletion in 15q11-q13, uniparental disomy 15, or a *UBE3A* mutation in AS. Approximately 2%–4% of patients have apparently normal chromosomes of biparental origin, but either the paternal chromosome carries a maternal imprint or the maternal chromosome carries a paternal imprint. In some of these cases, the incorrect epigenotype is the result of a putative imprint-switch failure in the parental germ line, caused by a microdeletion of the imprinting center (IC) (Reis et al. 1994; Sutcliffe et al. 1994; Buiting et al. 1995; Saitoh et al. 1996). The IC has been mapped to the *SNRPN* locus (fig. 1) and appears to have a bipartite structure (Buiting et al. 1995; Dittrich et al. 1996). In the PWS families, the smallest region of deletion overlap (PWS-SRO) is 4.3 kb and spans the *SNRPN* CpG island including exon 1 (Buiting et al. 1995; Saitoh et al. 1996; R. D. Nicholls, unpublished data). These deletions appear to block the maternal-to-paternal imprint switch in the paternal germ line (Dittrich et al. 1996). In the AS families, a 1.15-kb region (AS-SRO) immediately distal to an alternative 5′ exon of *SNRPN,* called "BD3" or "IC3," represents the shortest region of deletion overlap (Buiting et al. 1995; Saitoh et al. 1996; R. D. Nicholls, unpublished data). These deletions appear to block the paternal-to-maternal imprint switch in the maternal germ line (Dittrich et al. 1996).

Bürger et al. (1997) have recently shown that IC deletions cannot account for all imprinting defects in AS patients. Among nine families, only one family with two affected sibs had an IC deletion. No mutations were found in the other eight sporadic patients. In two of these families, the patient and a healthy sibling shared the same maternal alleles. In one of these families and in two others in which grandparental DNA samples were available, the incorrectly (paternally) imprinted maternal chromosome in the patient was found to be inherited from the maternal grandmother. The latter finding is not compatible with a paternal-to-maternal imprint-switch failure in the maternal germ line. We have extended this analysis to additional non–IC-deletion AS patients and—for the first time—to a large series of non–IC-deletion PWS patients. This analysis has important impli-

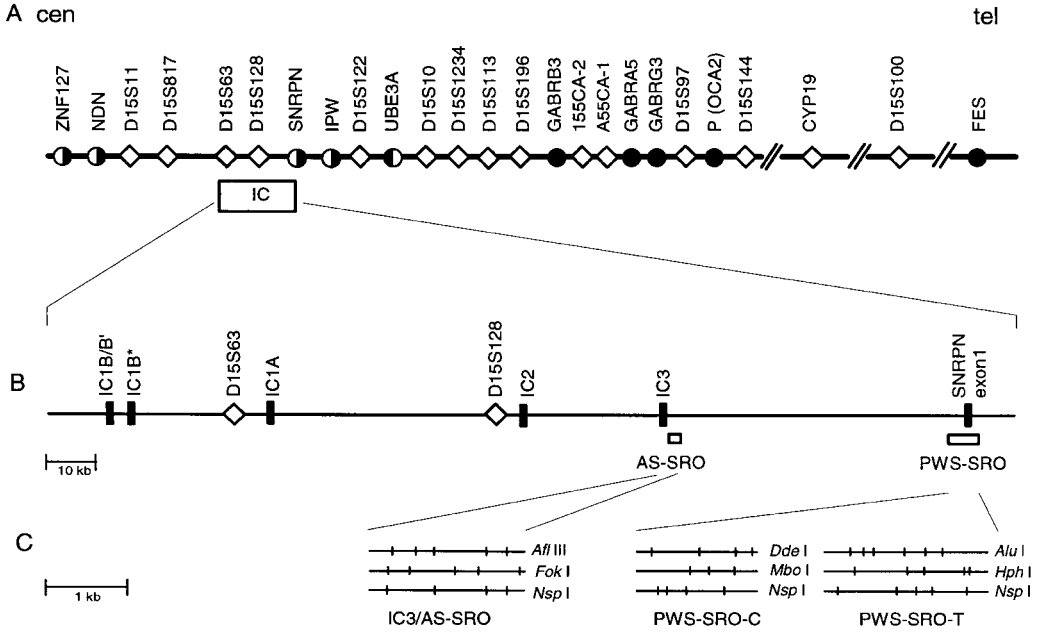

Figure 1 Overview of proximal 15q. *A,* Order of genes and anonymous DNA markers in proximal 15q. *B,* Physical map of the IC. *C,* Restriction map of the IC regions analyzed by heteroduplex analysis. Diamonds denote polymorphic DNA markers; half-blackened circles denote imprinted genes; blackened circles denote biallelically expressed genes; blackened boxes denote exons; unblackened boxes denote the IC and essential IC elements (AS-SRO and PWS-SRO).

cations for imprint switching and genetic counseling. Furthermore, we report the first two cases of prenatal diagnosis on the basis of epigenetic analysis.

Patients, Material, and Methods

All patients were seen by experienced clinicians and were diagnosed as having AS or PWS. Patients ASID-06 and ASID-16 were originally suspected of having PWS, because of obesity that started during early childhood. These two patients may belong to a subgroup of AS imprinting-defect patients whose phenotype overlaps with PWS (G. Gillessen-Kaesbach, unpublished data). The integrity of the IC in patients ASID-01 and ASID-02 was described by Bürger et al. (1997). The grandpaternal origin of the incorrectly imprinted chromosome in patient PWSID-04 was described by Schulze et al. (1997). Patients ASID-08 and ASID-09 are individuals VIII-5 and VIII-9 in the report by Beuten et al. (1996). They are distantly related, but the chromosome 15q11-q13 haplotypes are different, suggesting that independent events gave rise to AS in these patients (Beuten et al. 1996). In each case, informed consent was obtained from the parents. The study was approved by the local ethics committee.

Genomic DNA was extracted from peripheral blood leukocytes by use of standard procedures. Methylation at the *D15S63* (PW71) and *SNRPN* loci was investigated by Southern blot analysis with probes PW71B (*Cfo*I + *Bgl*II) (Dittrich et al. 1992) and kb17 (*Bgl*II + *Not*I or *Bgl*II + *Hpa*II) (Saitoh et al. 1997; K. Buiting, unpublished data) or by the recently described methylation-specific *SNRPN* PCR test (Kubota et al. 1997; Zeschnigk et al. 1997). Deletion screening was performed by means of quantitative Southern blot analysis with a battery of probes from the IC (Buiting et al. 1995). Three micrograms of DNA were digested with appropriate restriction enzymes (New England Biolabs or Boehringer Mannheim), resolved on 0.7% or 1.0% agarose gels, and analyzed by Southern blot hybridization. Probes were labeled by means of random oligonucleotide priming and α-[^{32}P] CTP (NEN Dupont). Autoradiography was performed at −80°C with intensifying screens and Kodak XAR films.

Genotypes were determined at the following microsatellite loci: D15S817 (Genome Database 1998), D15S11 (Mutirangura et al. 1992*a*), D15S63 (Wagstaff et al. 1993), D15S128 (Gyapay et al. 1994), D15S1234 and D15S196 (Trent et al. 1995), D15S10 (Lindeman et al. 1991), D15S113 (Mutirangura et al. 1993), LS6-2 (S. Christian, personal communication), GABRB3 (Mutirangura et al. 1992*b*), 155CA-2 and A55CA-1 (Glatt et al. 1994), GABRA5 (Glatt et al. 1992), D15S97 (Beckmann et al. 1993), D15S144 (Gyapay et al. 1994), CYP19 (Polymeropoulos et al. 1991*b*), D15S100 (Hudson et al. 1992), and FES (Polymeropoulos et al. 1991*a*). PCR products were labeled with either fluorochromes (Applied Biosystems) or ^{32}P and were analyzed by standard methods.

The IC exons (Dittrich et al. 1996) were PCR amplified and sequenced on both strands, as described by Bürger et al. (1997). The IC3/AS-SRO and the PWS-SRO were analyzed by heteroduplex analysis. The IC3/AS-SRO (nucleotides −56 to +1840, where +1 is the first nucleotide of exon IC3) was amplified with primers ExBD3x, 5′-GTA CTT CTA TTT TGA ATG ACC-3′, and IC3, 5′-AGT GGC TGA TAC AGA ATA AG-3′ (annealing temperature 56°C). The PWS-SRO was divided into two subregions, which are separated by an *Alu* repeat: PWS-SRO–C (nucleotides −3185 to −1714, where +1 is the first nucleotide of *SNRPN* exon 1) was amplified with primers RN428, 5′-CAG CCA AGT ACT AAC ACT TC-3′, and RN435, 5′-GAA CAG GTC CTA GTA TAA GC-3′ (annealing temperature 62°C). PWS-SRO–T (nucleotides −1357 to +538) was amplified with primers RN436, 5′-TTT CGA TTG ACT CCC GTG AT-3′, and PWSSRO2, 5′-CGA TCA CTT CAC GTA CCT TC-3′ (annealing temperature 58° C). In each case, 35 cycles of denaturation (15 s at 95°C), annealing (30 s), and elongation (30 s at 72°C) in a Perkin Elmer 9600 Thermal Cycler were used. To obtain smaller fragments for heteroduplex analysis, aliquots of the IC3/AS-SRO PCR product were digested with *Afl*III, *Fok*I, and *Nsp*I (see restriction map, fig. 1*C*). The PWS-SRO–C PCR product was digested with *Dde*I, *Mbo*II, and *Nsp*I, and the PWS-SRO–T PCR product was digested with *Alu*I, *Hph*I, and *Nsp*I (fig. 1*C*). The digestion products were subjected to heteroduplex analysis on high-resolution polyacrylamide gels, as described by Lohmann et al. (1996). Abnormal bands were sequenced. Because of the limited amounts of DNA, the PWS-SRO–T region in patient PWSID-13 and both PWS-SRO subregions in patient PWSID-09 could not be analyzed.

Results

In this study, we investigated 13 PWS patients and 17 AS patients who, according to microsatellite analysis, had apparently normal chromosomes 15 of biparental origin. Methylation analysis of *D15S63* and *SNRPN* revealed that the PWS patients had a maternal methylation pattern and the AS patients had a paternal methylation pattern on both chromosomes. The parents had a normal methylation pattern (data not shown). These findings classify the patients as having an imprinting defect. Apart from patient PWSID-13, who has an affected MZ twin sister, none of the patients has an affected sib (table 1).

In contrast to several other patients with an imprinting defect (Reis et al. 1994; Sutcliffe et al. 1994; Buiting et

Table 1

Patient Characteristics

Patient	Referring Lab	Number of Affected Sibs	Number of Unaffected Sibs/Unaffected Sibs with the Same IC Haplotype	IC Deletion	Origin of Incorrectly Imprinted Chromosome Region (Method)
PWSID-01	Bristol	0	1/nt	−	ni
PWSID-02	Hamburg	0	1/nt	−	ni
PWSID-03	Espinardo	0	2/nt	−	PGM (MS)
PWSID-04	Glostrup	0	1/ni[a]	−	PGM (MS)[a]
PWSID-05	Essen	0	1/1	−	PGM (RM)
PWSID-06	Heidelberg	0	1/nt	−	PGM (MS)
PWSID-07	Jerusalem	0	0	−	ni
PWSID-08	Jerusalem	0	2/nt	−	nt
PWSID-09	Sydney	0	1/nt	−	ni
PWSID-10	Sydney	0	2/nt	−	ni
PWSID-11	Heidelberg	0	0	−	PGM (MS)
PWSID-12	Maastricht	0	1/nt	−	PGM (MS)
PWSID-13	Rotterdam	1[b]	1/1	−	ni
ASID-01	Berlin	0	1/1[c]	−[c]	MGF (RM)
ASID-02	Berlin/Bonn	0	2/2[d]	−[e]	ni
ASID-03	London	0	2/nt	−	nt
ASID-04	Loverval	0	0	−	ni
ASID-05	Loverval	0	0	−	ni
ASID-06	Leuven	0	2/nt	−	ni
ASID-07	Antwerp	0	?	−	MGM (RM)
ASID-08[f]	Antwerp	0	3/nt	−	ni
ASID-09[f]	Antwerp	0	1/nt	−	MGF (RM)
ASID-10	Oulu	0	1/0	−	ni
ASID-11	Boston	0	?	−	nt
ASID-12	Boston	0	?	−	nt
ASID-13	Sydney	0	1/nt	−	MGF (RM)
ASID-14	Sydney	0	3/nt	−	MGF (RM)
ASID-15	Sydney	0	2/nt	−	ni
ASID-16	Essen	0	1/0	−	MGM (MS)
ASID-17	Adelaide	0	1/0	−	MGF (MS)

NOTE.—nt = not tested; ni = not informative; PGM = paternal grandmother; MGM = maternal grandmother; MGF = maternal grandfather; MS = microsatellite analysis; and RM = RFLP/methylation analysis of *SNRPN*. A minus sign (−) indicates absence, and a question mark (?) indicates unknown.

[a] Reported by Schulze et al. (1997).

[b] This is an MZ twin.

[c] Reported by Bürger et al. (1997). ASID-01 is patient K in the previous publication.

[d] These are two aborted fetuses who had a normal methylation pattern (see text for details). A pathological examination was not performed.

[e] The integrity of the IC was reported by Bürger et al. (1997). ASID-02 is patient B in the previous publication.

[f] ASID-08 and ASID-09 are individuals VIII-5 and VIII-9 in the report by Beuten et al. (1996).

al. 1995; Saitoh et al. 1996; Schuffenhauer et al. 1996), Southern blot analysis with probes from the IC did not reveal any qualitative or quantitative changes (data not shown). To search for very small deletions and point mutations, we screened the IC3/AS-SRO in the AS patients and the PWS-SRO in the PWS patients (figs. 1*B* and *C*) by heteroduplex analysis, which detects >95% of small insertions and deletions and >70% of single-nucleotide exchanges (Lohmann et al. 1996). Furthermore, we sequenced all the IC exons. Apart from DNA polymorphisms (to be reported elsewhere), we did not detect any structural alterations.

To determine the grandparental origin of the incorrectly imprinted chromosome, we genotyped available family members by means of microsatellite analysis (fig. 2) and combined RFLP/methylation analysis of the *SNRPN* locus (fig. 3). The latter test (Saitoh et al. 1997) makes it possible to determine the grandparental origin of the chromosomes 15 in the absence of grandparental DNA samples, provided the family is informative for the *Hpa*II/*Msp*I RFLP in intron 1 of *SNRPN* (for details, see the legend to fig. 3). Seven families with an AS child and five families with a PWS child were informative by one or the other method. In the PWS patients, the paternal chromosome carrying a maternal imprint was always derived from the paternal grandmother. In the AS patients, the maternal chromosome carrying a paternal imprint was inherited from the maternal grandfather in

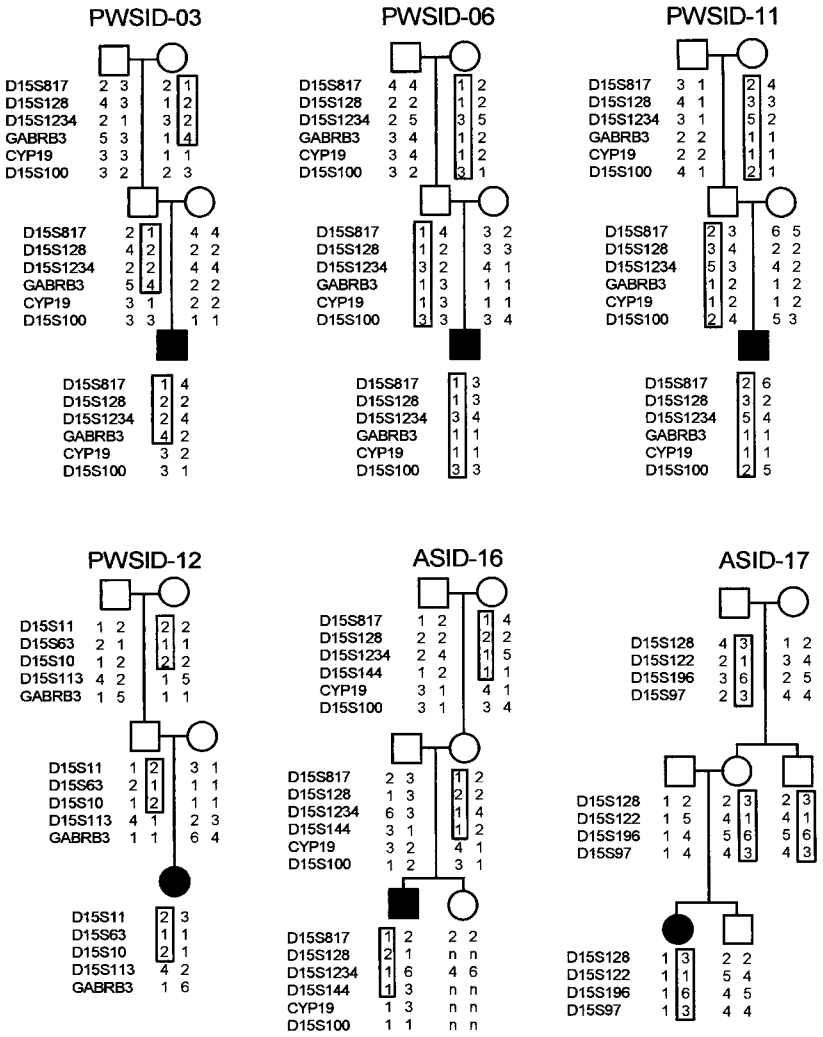

Figure 2 Segregation of 15q11-q13 haplotypes. The numbering of microsatellite alleles is by increasing size of the PCR products in each family ("n" = not tested).

five cases and from the maternal grandmother in two cases (table 1).

During the course of this study, the parents of patient ASID-02, patient PWSID-05, and patient PWSID-13 conceived another child and asked for prenatal diagnosis. In the first two families, microsatellite analysis of a chorionic villus sample (CVS) revealed that, in the IC region, the fetus had the same genotype as the affected child (figs. 4*A* and *B*). In family PWSID-13, the fetus shared the paternal haplotype with the affected MZ twins (fig. 4*C*). At the time of testing family ASID-02, no methylation data on CVS DNA were available, and the parents opted for an abortion. The DNA obtained from brain and liver of the aborted fetus was partially degraded and could not be analyzed by a methylation-specific Southern blot test. However, when methylation data on fetal DNA samples (Kubota et al. 1996) and a methylation-specific PCR test became available (Kubota et al. 1997; Zeschnigk et al. 1997), we reinvestigated this case and found a normal methylation pattern (fig. 4*A*). In the next pregnancy, a spontaneous abortion occurred 1 d prior to the scheduled CVS. We obtained a villus sample from the aborted material and found a normal methylation pattern (fig. 4*A*). In families PWSID-05 and PWSID-13, *SNRPN* methylation analysis of the chorion DNA revealed a normal pattern (figs. 4*B* and *C*). The parents decided to continue the pregnancy, and in each case a healthy child was born.

Discussion

This is the first comprehensive molecular study of AS *and* PWS patients who have an imprinting defect but no apparent structural alteration of the IC. By means of

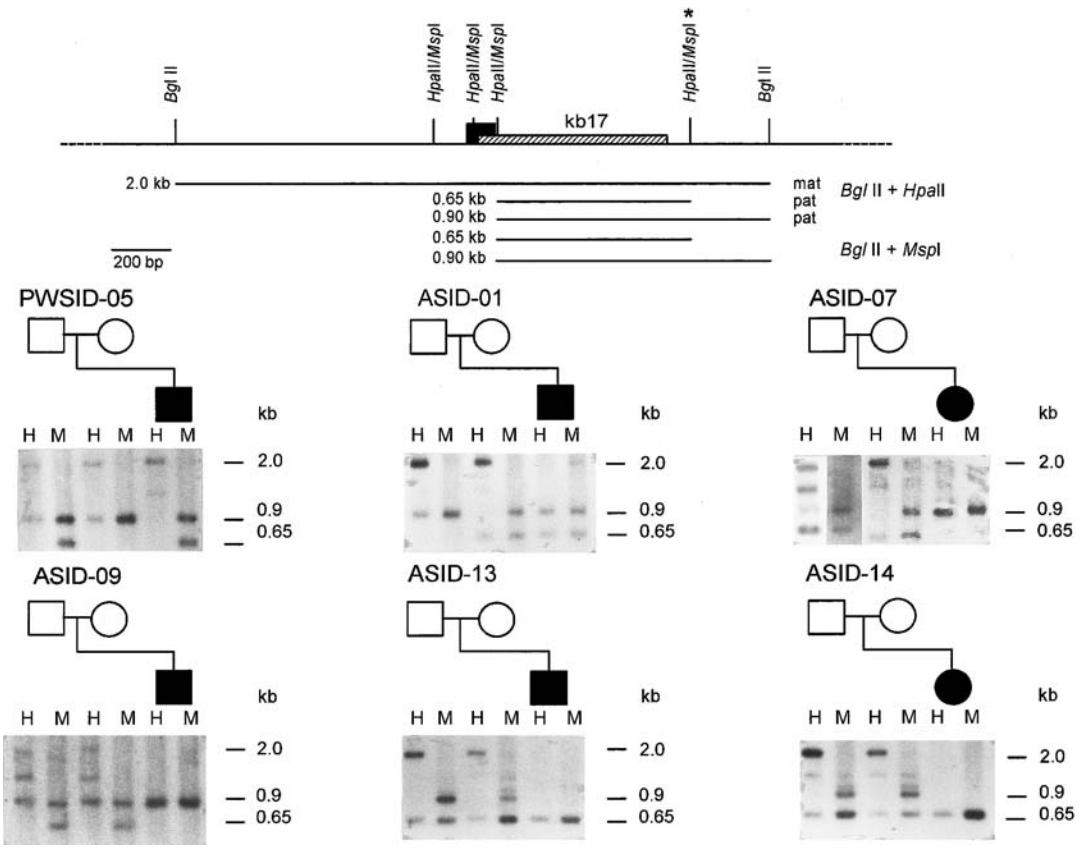

Figure 3 RFLP/methylation analysis of the *SNRPN* locus. DNA was digested with *Bgl*II+*Hpa*II (H) and *Bgl*II+*Msp*I (M) and hybridized with probe kb17 (*hatched box in the upper part of the figure*). *Hpa*II and *Msp*I have the same recognition sequence (CCGG), but *Hpa*II does not cleave methylated DNA. A variable *Msp*I/*Hpa*II site distal to *SNRPN* exon 1 (*blackened box*) and to kb17 is indicated by an asterisk (*). In informative families, comparison of the band pattern produced by *Msp*I with that produced by *Hpa*II allows inference of parental origin, since, in normal individuals, the paternal origin ("pat") is given by the unmethylated *Hpa*II allele (0.65 or 0.9 kb) and the maternal origin ("mat") is given by the methylated *Hpa*II allele (2.0 kb). In some *Hpa*II digests, an additional band of 1.3 kb is observed. This band may result from incomplete DNA methylation but does not interfere with the analysis. The results obtained from six informative families are shown in the lower part of the figure. Patient PWSID-05 is heterozygous for the 0.9-kb and 0.65-kb *Msp*I bands. As is obvious from the *Hpa*II digestion, both alleles are methylated, confirming that the paternal chromosome is incorrectly (maternally) imprinted. The 0.65-kb allele is derived from the father. In the father, who has a normal methylation pattern, the 0.65-kb allele is methylated and therefore of grandmaternal origin. Patient ASID-01, also, is heterozygous for the 0.9-kb and 0.65-kb *Msp*I bands. Both alleles are unmethylated, because the maternal chromosome is aberrantly (paternally) imprinted. The 0.65-kb allele is derived from the mother. In the mother, this allele is unmethylated and therefore of grandpaternal origin. Patient ASID-07 is homozygous for the 0.9-kb band. In the mother, the 0.9-kb band is methylated and therefore of grandmaternal origin. Patient ASID-09 is homozygous for the 0.9-kb band. In the mother, the 0.9-kb band is unmethylated and therefore of grandpaternal origin. Patients ASID-13 and ASID-14 are homozygous for the 0.65-kb band. In the mother, the 0.65-kb band is unmethylated and therefore of grandpaternal origin.

comparisons of the AS patients with the PWS patients and of this group of patients with AS and PWS patients who have an IC deletion, two important points emerge. First, all non–IC-deletion patients described here and elsewhere (Bürger et al. 1997) represent sporadic cases. In this context, patient PWSID-13 and her affected sister are treated as one case, because they are MZ twins. Furthermore, some patients share the maternal (AS) or the paternal (PWS) haplotype with an unaffected sib. These are patients PWSID-05 and PWSID-13 (present study), PWS-B (R. D. Nicholls, unpublished data), AS-Gr and AS-K (Bürger et al. 1997), and ASID-02 (present study). It is possible that there are more such cases, because most of the sibs were unavailable for testing (table 1). The findings are in marked contrast to patients with an IC deletion. As shown in table 2, all affected sib pairs analyzed to date have an IC deletion, and three of the four sporadic IC-deletion patients have an inherited deletion, which is associated with a 50% recurrence risk. Incorrect imprinting in the non–IC-deletion patients is probably the result of a spontaneous pre- or postzygotic error, which may be a *cis*-acting mutation outside of the

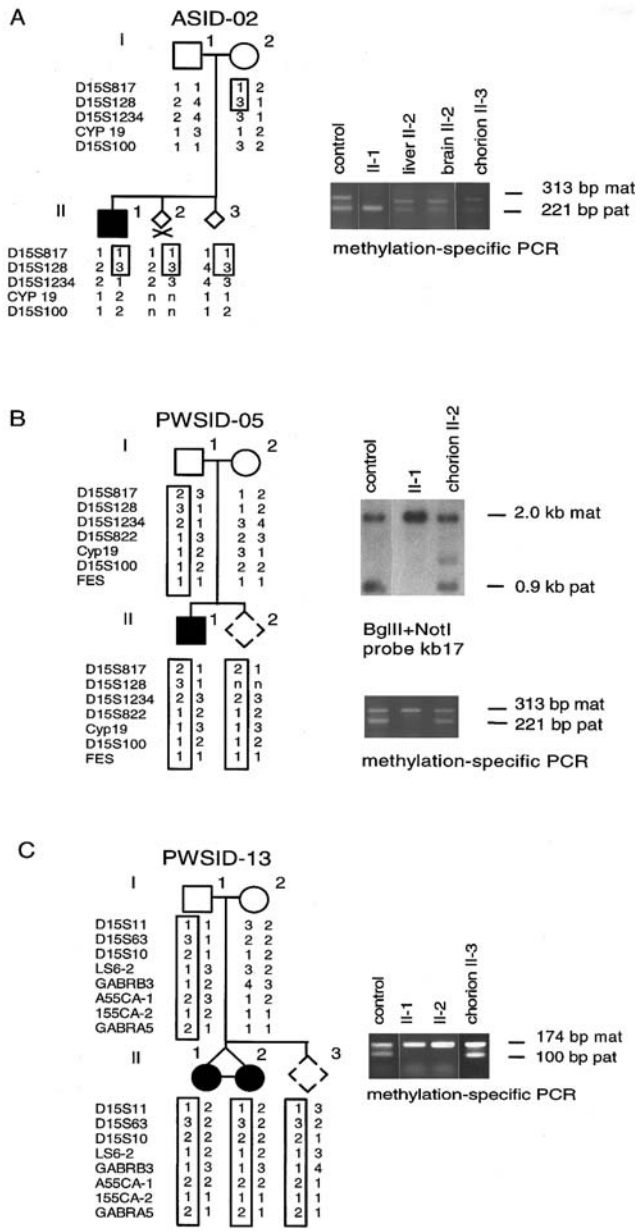

Figure 4 Prenatal diagnosis in families ASID-02 (*A*), PWSID-05 (*B*), and PWSID-13 (*C*). *A,* Owing to crossovers distal to D15S128, the haplotypes shown in the figure represent only one of several possibilities. However, the patient and the two aborted fetuses share the same maternal haplotype in the IC region. In the methylation-specific PCR test, the patient lacks the maternal band, whereas the fetal DNA samples have a normal methylation pattern. *B,* The patient and the fetus have the same 15q11-q13 genotype. In the methylation-specific Southern and PCR tests (Zeschnigk et al. 1997), the patient lacks the paternal band, whereas the fetus has a normal methylation pattern. *C,* The patients and the fetus have the same paternal 15q11-q13 haplotype. In the methylation-specific PCR test (Kubota et al. 1997), the affected twins lack the paternal band, whereas the fetus has a normal methylation pattern ("n" = not tested).

Table 2

IC Deletions in Sibs and Sporadic Patients with AS or PWS

	No. with AS		No. with PWS	
Molecular Class	Sibs	Sporadic Case	Sibs	Sporadic Case
IC deletion	6[a]	2[b]	6[c]	2[d]
No IC deletion detected	…	23[e]	…	18[f]

[a] Patient AS-D (Buiting et al. 1995); patients AS-R, AS-J, and AS-H (Saitoh et al. 1996); patient AS-F (K. Buiting and H. Kokkonen, unpublished data); and patient AS-O (R.D. Nicholls, unpublished data).

[b] Patients AS-C and AS-SCH. Both deletions are maternally inherited, and the parents have a 50% recurrence risk (Buiting et al. 1995; Saitoh et al. 1996).

[c] Patients PWS-O (Sutcliffe et al. 1994); PWS-S and PWS-U (Buiting et al. 1995); PWS-T (Teshima et al. 1996); and PWS-J and PWS-P (R. D. Nicholls, unpublished data).

[d] Patients PWS-KT and PWS-14. The deletion in PWS-KT is paternally inherited, and the parents have a 50% recurrence risk (Schuffenhauer et al. 1996). The deletion in PWS-14 occurred de novo on a paternal chromosome (R. D. Nicholls, unpublished data).

[e] Patients Ki, Gr, La, K, W, B, Ge, and Le (Bürger et al. 1997) and ASID-03–ASID-017 (present study). Three patients share the maternal haplotype with an unaffected sib: patients K and Gr (Bürger et al. 1997) and patient ASID-02 (present study).

[f] Patients PWSID-04 (Schulze et al. 1997), PWSID-01–PWSID-03 and PWSID-05–PWSID-13 (present study), PWS-B, PWS-G, and PWS-29 (R. D. Nicholls, unpublished data), and Go and Ju (T. Buchholz, unpublished data). Three patients share the paternal haplotype with an unaffected sib: patients PWSID-05 and PWSID-13 (present study) and patient PWS-B (R. D. Nicholls, unpublished data).

surveyed regions of the IC, an epimutation resulting from erroneous imprinting or loss of imprinting, a paramutation resulting from interchromosomal transfer of epigenetic states, or a mutation in a *trans*-acting factor (for a more detailed discussion of these mechanisms, see Bürger et al. 1997).

Second, Bürger et al. (1997) showed that, in three non–IC-deletion AS patients, the incorrectly imprinted chromosome was derived from the maternal grandmother. This is in marked contrast to AS IC deletions, which are always on the chromosome inherited from the maternal grandfather (table 3). Here we confirm the findings that, in AS, the incorrectly imprinted chromosome can be derived from either the maternal grandfather or the maternal grandmother, but, most importantly, we demonstrate that the situation in PWS is not reciprocal to that in AS (table 3). In each of five non–IC-deletion PWS patients reported here and four non–IC-deletion PWS patients described elsewhere (Schulze et al. 1997; R. D. Nicholls, unpublished data; T. Buchholz, unpublished data), the incorrectly imprinted chromosome is derived from the paternal grandmother, as is the case in all IC-deletion patients (table 3). This is significantly different from equal grandpaternal and grandmaternal inheritance (2 df, $P = .0039$).

Table 3

Grandparental Origin of the Aberrantly Imprinted Chromosome Region

	No. of Patients with AS		No. of Patients with PWS	
Origin	IC Mutation	No IC Mutation	IC Mutation	No IC Mutation
Maternal grandfather	5[a]	5[b]	…	…
Maternal grandmother	…	5[c]	…	…
Paternal grandfather	…	…	…	…
Paternal grandmother	…	…	6[d]	9[e,f]

[a] Patients AS-C and AS-D (Buiting et al. 1995), AS-SCH (Saitoh et al. 1996), AS-F (K. Buiting and H. Kokkonen, unpublished data), and AS-O (R. D. Nicholls, unpublished data).

[b] Patients ASID-01, ASID-08, ASID-13, ASID-14, and ASID-17 (present study).

[c] Patients La, Ki, and Gr (Bürger et al. 1997); patients ASID-07 and ASID-16 (present study).

[d] Patient PWS-O (Sutcliffe et al. 1994), patients PWS-U and PWS-S (Buiting et al. 1995), patient PWS-KT (Schuffenhauer et al. 1996), and patients PWS-P and PWS-T (R. D. Nicholls, unpublished data).

[e] Patients PWSID-3, PWSID-05, PWSID-06, PWSID-11, and PWSID-12 (present study); patient PWSID-04 (Schulze et al. 1997); patient PWS-B (R. D. Nicholls, unpublished data); and patients Go and Ju (T. Buchholz, unpublished data).

[f] Significantly different from equal grandpaternal and grandmaternal inheritance (2 df, $P = .0039$).

These results have important implications for understanding the mechanism of imprint switching. The finding of kilobase-sized IC deletions in all affected sib pairs and the lack of finding point mutations in the PWS-SRO and AS-SRO IC regions in the other patients suggest either that the *cis*-acting imprint-switch elements may not be strictly sequence dependent or that there are multiple, partially redundant elements within each SRO. We note that, in this respect, the silencer activity of the PWS-SRO in transgenic *Drosophila,* which may be related to the imprint-switch activity of this region or to *SNRPN* transcriptional regulation in humans, is size dependent (Lyko et al. 1998). Although the silencer activity could be narrowed down to a 215-bp region overlapping the *SNRPN* promoter, larger fragments of the PWS-SRO gave a much stronger effect. Thus, point mutations or deletions of only a few base pairs may not be sufficient to impair imprint switching and therefore may not be observed in typical PWS and AS patients with an imprinting defect.

If the sporadic imprinting defects arise prezygotically, we will have to accept the idea that the female germ line is capable of making a paternal imprint, because, in five of nine AS patients, the incorrectly (paternally) imprinted maternal chromosome was inherited from the maternal grandmother (table 3). As judged from the methylation analysis of sites normally methylated on the paternal chromosome (e.g., intron 7 of *SNRPN;* Glenn et al. 1993; Buiting et al. 1994), the patients do not have an imprint-free maternal chromosome but a paternally imprinted maternal chromosome (B. Dittrich and C. Färber, unpublished data). Thus, the (grand)maternal imprint was erased in the maternal germ line, and a paternal imprint developed. As shown in table 3, we have not observed the reciprocal situation in PWS—that is, in PWS patients in whom the incorrectly (maternally) imprinted paternal chromosome was inherited from the paternal grandfather. Although the number of PWS patients studied to date ($n = 9$) is too small to rule out such a case, this finding suggests that the (grand)maternal imprint was not erased in the paternal germ line rather than established de novo. On the basis of the nonreciprocal findings in AS and PWS, we suggest that the paternal imprint may be the default imprint, which develops in the paternal and the maternal germ line, if either a maternal *trans*-acting factor is missing (male germ line) or maternal imprinting fails (maternal germ line of AS patients with an imprinting defect). Recent experiments with chimeric mice made with embryonic germ (EG) cells also suggest that the paternal imprint is the default state. As described by Tada et al. (1998), EG cells from both male and female mouse embryos have an equivalent epigenotype. Most strikingly, both alleles of the *p57kip2* gene, which is maternally expressed and paternally methylated, were undermethylated in EG cells from male and female embryos but underwent de novo methylation in primary embryonic fibroblasts rescued from EG-cell chimeras, to resemble a paternal allele in somatic cells.

If the parental imprints are erased in the germ line (Ferguson-Smith 1996; Shemer et al. 1997) and the paternal imprint is the default state, the imprint-switch hypothesis (Dittrich et al. 1996) can be extended as follows (fig. 5): Imprint erasure (at least erasure of the maternal imprint) requires in *cis* an element in the PWS-SRO of the IC. In the absence of this element (PWS patients with an IC deletion), the maternal imprint on the grandmaternal chromosome is not erased in the paternal germ line and transmitted to the patient. In non–IC-deletion, PWS patients, imprint erasure in the

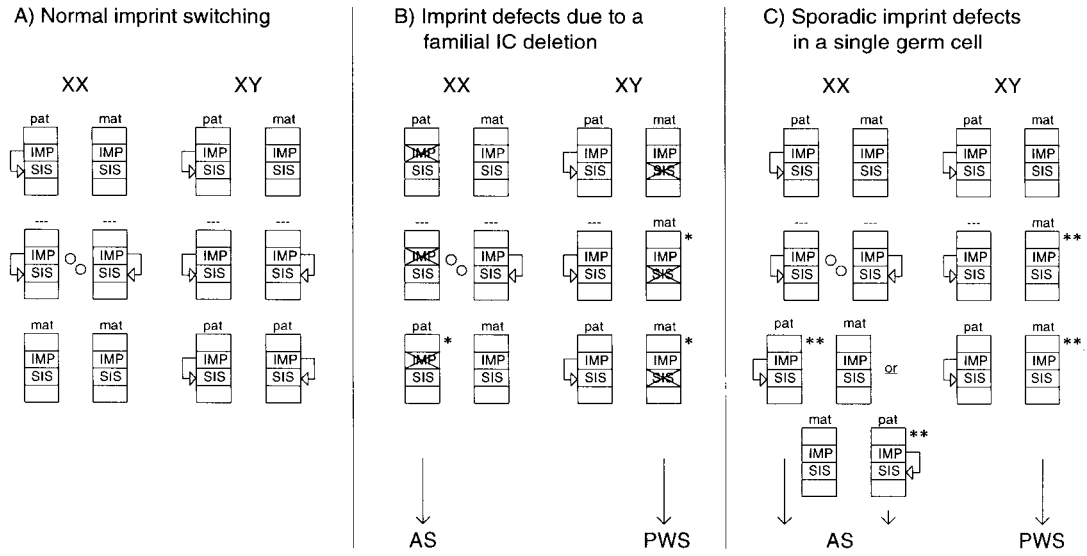

Figure 5 Imprint switching and imprinting defects in proximal 15q. The figure shows the imprinted domain on the two chromosomes 15 (*boxes*) in somatic cells (*upper row*), primordial germ cells (*middle row*), and germ cells (*lower row*). *A,* Normal imprint switching. The paternal imprint ("pat") and the maternal imprint ("mat") are erased (---) in primordial germ cells and reset according to the sex of the individual (XX or XY). Imprint erasure and resetting is initiated at the switch initiation site (SIS = PWS-SRO). Maternal imprinting requires imprintor (IMP = IC3/AS-SRO) activity in *cis* (*open arrow*) and an XX-specific factor in *trans* (*circles*). In the absence of the *trans*-factor (XY germ line), a paternal imprint develops. *B,* Deletion of the imprintor prevents maternal imprinting in the female germ line, and a paternal imprint develops by default. Children inheriting this chromosome will develop AS. A deletion of the switch initiation site blocks the erasure of the maternal imprint in the paternal germ line, and children inheriting this chromosome will develop PWS. In both cases, the recurrence risk is 50%. *C,* Sporadic imprinting defects in a single germ cell. Failure of a single maternal germ cell to establish a maternal imprint on the paternally or the maternally derived chromosome leads to the development of a paternal imprint on either chromosome by default. Failure of a single paternal germ cell to erase the maternal imprint on the maternal chromosome leads to a paternal chromosome with a maternal imprint. The recurrence risk is most likely not increased. The symbol " × " indicates deletion, an asterisk (*) indicates an incorrectly imprinted chromosome, and a double asterisk (**) indicates that only one germ cell contains an incorrectly imprinted chromosome; the other germ cells have correctly imprinted chromosomes.

paternal germ line failed for unknown reasons. Maternal imprinting requires the IC3/AS-SRO in *cis* and an XX-specific factor in *trans*. In the absence of the *cis*-acting element (AS patients with an IC deletion), or if the maternal imprinting fails for other reasons (AS patients without an IC deletion), the erased chromosome acquires a paternal imprint by default and is transmitted to the patient. Note that sporadic imprinting defects may arise not only by the mechanism shown in figure 5*C* but also by a prezygotic or postzygotic epi- or paramutation; for example, loss of the maternal imprint during postzygotic cell divisions may lead to AS (see discussion above; Bürger et al. 1997).

The results reported or reviewed here establish that AS and PWS patients with an imprinting defect fall into two molecular classes: those with an IC deletion and those without an apparent IC deletion. This distinction has important implications for genetic counseling. Families with an IC deletion have a recurrence risk of 50% if one of the parents carries the deletion. In the case of mosaicism, the recurrence risk depends on the ratio of mutant to normal germ cells (Saitoh et al. 1996; Bürger et al. 1997). Families without an IC deletion would be expected to have a low recurrence risk, as in the families reported here. In AS, inheritance of the incorrectly imprinted chromosome from the maternal grandmother appears to be an additional indicator for a low recurrence risk, because, in all affected sib pairs studied to date, it was the grandpaternal chromosome that was incorrectly imprinted (tables 2 and 3). However, as we do not yet understand the cause of incorrect imprinting in non–IC-deletion PWS and AS, a recurrence risk cannot absolutely be excluded, and prenatal diagnosis should be offered to such families.

In non–IC-deletion cases, a safe prenatal diagnosis can be based only on methylation analysis because affected and unaffected sibs may share the same haplotype. The *D15S63* (PW71) test cannot be used for this purpose, because this locus is hypomethylated in extraembryonic tissue (Dittrich et al. 1993; Kubota et al. 1996). In contrast, the *SNRPN* methylation imprint is inherited from the gametes and maintained in embryonic and extraembryonic cell lineages (Glenn et al. 1996; Kubota et al. 1996; Shemer et al. 1997). Recently, a PCR version of

the *SNRPN* methylation test has become available (Kosaki et al. 1997; Kubota et al. 1997; Zeschnigk et al. 1997). As shown here, the PCR test also works on partially degraded DNA and was invaluable in demonstrating that the aborted fetus in family ASID-02, which had the same genotype as the patient, was unaffected by AS. This established that the recurrence risk in this family was low and encouraged the family to consider another child. In families PWSID-05 and PWSID-13, we also found that the fetus had a normal methylation pattern despite having the same paternal haplotype as the patient. We predicted that the fetuses would not be affected by PWS, and, in both cases, a healthy child was born. To the best of our knowledge, these are the first two cases of prenatal diagnosis based on epigenetic analysis.

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Electronic-Database Information

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

Genome Database, http://www.gdb.org (for microsatellite loci used to determine genotypes)

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

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