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Methylation analysis of the PWS/AS region does not support an enhancer-competition model

In a recent article in *Nature Genetics*[1], Tilghman *et al.* propose an enhancer-competition model to explain genomic imprinting in the Prader-Willi/Angelman syndrome (PWS/AS) region on human chromosome 15. The key elements of this model are as follows: (i) the minimal imprinting centre (IC) region deleted in AS patients with an imprinting defect (AS-SRO) is extensively methylated in the female germ line and induces post-fertilization spreading of DNA methylation along the maternal chromosome; (ii) the minimal IC region deleted in PWS patients with an imprinting defect (PWS-SRO), which contains the *SNRPN* promoter, functions in the soma to maintain the entire PWS region in an unmethylated state; (iii) *SNRPN* and the AS gene (*UBE3A*) compete for a neuronal enhancer. The authors claim that their model is simpler than the model proposed by Dittrich *et al.*[2], which suggests that maternal imprinting requires the AS-SRO in *cis* and an XX-specific factor in *trans*, and that the PWS-SRO is the 'imprint switch initiation site'.

We have investigated the methylation status of the AS-SRO, which is 1.15 kb in size and contains 16 CpG dinucleotides (ref. 3; Fig. 1*a*,*b*). As shown by Southern analysis with the methylation-sensitive restriction enzymes *Hpa*II and *Cfo*I (Fig. 1*c*,*d*) and by sequencing of bisulfite-treated genomic DNA (Fig. 2), the AS-SRO is extensively methylated (83–87%) on both maternal and paternal chromosomes in healthy (Fig. 2*a*,*b*), PWS (Fig. 2*c*) and AS (Fig. 2*d*) individuals. There is a low degree of mosaicism, but no parent-of-origin specific methylation. Thus, the central statement of Tilghman *et al.* claiming that the AS-SRO region is specifically methylated in the maternal germ line is not in agreement with experimental results. In contrast, Shemer *et al.*[4] have obtained experimental evidence that differential methylation of *SNRPN* represents the primary epigenetic mark.

Furthermore, Glenn *et al.*[5] and Buiting *et al.*[6] have demonstrated that CpG dinucleotides in intron 7 of the human *SNRPN* gene and 15 kb upstream of the AS-SRO (Y48.5) are methylated on the paternal

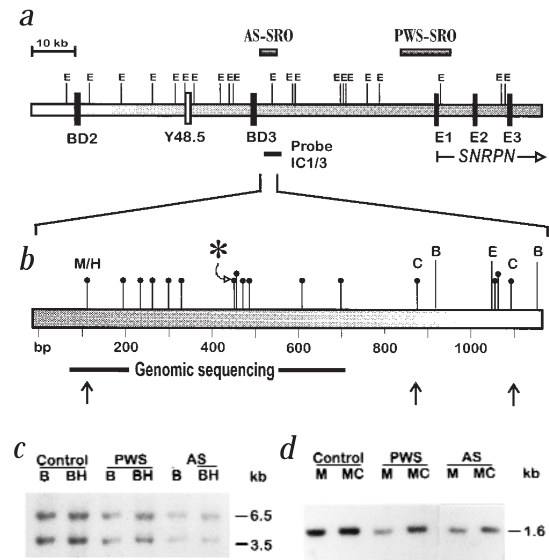

Fig. 1 Physical map of the imprinting centre and methylation status of *Hpa*II and *Cfo*I sites. ***a***, *Eco*RI (E) restriction map. Filled boxes, exons; open box, Y48.5. ***b***, Physical map. The AS-SRO contains 16 CpGs (filled circles), one of which is polymorphic (*). CpGs indicated by an arrow are part of *Msp*I/*Hpa*II (M/H) or *Cfo*I (C) sites. ***c***, Methylation analysis of the *Hpa*II site. DNA samples from normal controls, PWS patients with a paternal deletion 15q11–q13 and AS patients with a maternal deletion 15q11–q13 were cleaved with *Bgl*II (B) or *Bgl*II+*Hpa*II (BH) and probed with IC1/3. In each of the DNA samples, *Bgl*II fragments were resistant to cleavage by *Hpa*II. ***d***, After cleavage with *Msp*I (M) or *Msp*I+*Cfo*I (MC), the probe detected the same fragment in each of the samples, in other words, both *Cfo*I sites were resistant to cleavage.

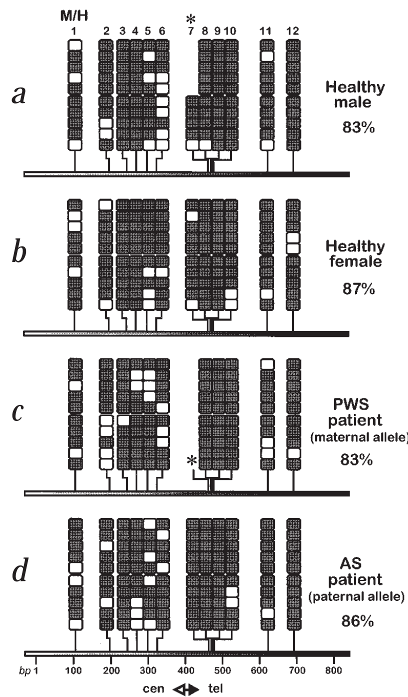

Fig. 2 Methylation status of the AS-SRO as determined by genomic sequencing. Two healthy individuals (***a***,***b***), one PWS patient with a paternal deletion 15q11–q13 (***c***) and one AS patient with a maternal deletion 15q11–q13 (***d***) were studied. After bisulfite treatment of the DNA, which converts C, but not 5-methyl-C, to uracil[9,10], the segment designated 'Genomic sequencing' (Fig. 1*b*) was amplified by nested PCR with primers 1A and1B, and primers 2A and 2B (1A, 5´–GATTTAATTGTATAATTTAATTATTTT-TTTAT–3´; 1B, 5´–CCTCCAACACATACTCTTTCAATC-ATTATCTAC–3´; 2A, 5´–ATTTTTTTATTTTTTGGATAT-AGTTTTTTTTTT–3´; 2B, 5´–CTACTTAAAAAAAATACTT-CACCTAAATAACTC–3´) and cloned. For one strand of each sample, 10 clones were sequenced. Each horizontal row of squares presents the C/mC distribution in one clone (open square, C; closed square, mC). CpG 7 (*) is polymorphic and allows the two alleles in the healthy male to be distinguished. Numbers indicate percentage of methylation.

chromosome and undermethylated on the maternal chromosome. Paternal methylation of these sites and biallelic methylation of the AS-SRO does not support the second statement of Tilghman *et al.* claiming that the *SNRPN* promoter functions in the soma to maintain the entire PWS region in an unmethylated state.

The enhancer-competition model also fails to explain important genetic aspects. In AS patients, IC deletions (familial and *de novo*) are always on the chromosome inherited from the maternal grandfather and in PWS patients the IC deletions are always on the chromosome inherited from the paternal grandmother. Even in IC non-deletion PWS patients with an imprinting defect it is always the grandmaternal chromosome that is incorrectly imprinted[7]. The enhancer-competition model would predict that imprinting defects can occur on either grandparental chromosome. Furthermore, at least two PWS IC deletions, which lead to a maternal imprint on the paternal chromosome, include the AS-SRO (ref. 8). Thus, a maternal imprint has developed in the absence of the AS-SRO, although the enhancer-competition model postulates that this region bears the maternal epigenetic mark which induces spreading of DNA methylation along the chromosome.

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