

## Letter to the Editor

# Potential Pitfall in Prader-Willi Syndrome and Angelman Syndrome Molecular Diagnosis

### To the Editor:

Prader-Willi syndrome (PWS) and Angelman syndrome (AS) are distinct neurodevelopmental disorders [Holm et al., 1993; Williams et al., 1995] caused by lack of paternal or maternal contribution, respectively, of the chromosome 15q11-q13 region. Although they provide one of the best known examples of imprinting in the human genome [Driscoll et al., 1992], the mechanisms underlying genomic imprinting remain unclear, but DNA methylation is involved in this process [John and Surani, 1996]. To date, all PWS and AS patients characterized by deletion, uniparental disomy, or mutation in the imprinting center have shown a parent-of-origin specific DNA methylation in 15q11-q13. According to the ASHG/ACMG report [1996] the molecular diagnosis of PWS and AS is based on methylation analysis by Southern blot using either PW71B (locus D15S63) or SNRPN probes, although these analyses do not discriminate between the three major classes of genetic defects. On the basis of reported cases, this test allows the detection of almost all PWS patients [Schulze et al., 1996] and about 80% of AS patients [Kishino et al., 1997; Matsuura et al., 1997]. PWS-affected individuals only display the single methylated maternal allele, whereas AS affected individuals only present the single unmethylated paternal allele at both PW71 and SNRPN loci. Here we describe four patients, three referred as AS and one as PWS, displaying an unusual PW71B pattern and a normal biparental SNRPN pattern.

For Southern blotting, genomic DNA was isolated from peripheral leukocytes and digested by either *Bgl*II and *Cfo*I enzymes for the PW71B probe described by Dittrich et al. [1993] or *Xba*I and *Not*I enzymes for the 0.6 kb SNRPN probe described by Glenn et al. [1996]. Methylation analysis with PW71B probe detects two bands in unaffected individuals of 8.0 and 6.4 kb [Gillissen-Kaesbach et al., 1995], whereas PWS patients only exhibit the maternal band (8.0 kb), and AS patients only show the paternal band (6.4 kb). Among

120 patients referred for PWS or AS molecular diagnosis, we found four children showing a normal methylation pattern with paternal and maternal allele at the SNRPN locus (data not shown) and an unusual methylation pattern at the PW71B locus (Fig. 1). Two different profiles were found in these patients. Southern blot analyses performed on DNA from the parents, using *Bgl*II and *Cfo*I, revealed that in each family one parent presented a normal profile whereas the other parent displayed an unexpected profile that was either identical or different from the one observed in the child (Fig. 1a, c, e, g). The finding of an additional pattern for the father in family 4 led to 3, the number of profiles.

To check whether these unexpected patterns were because of a deletion, a particular methylation imprint, or a simple restriction polymorphism, extended analyses were performed by PW71B Southern blot using a set of additional restriction enzymes [Dittrich et al., 1993]. Single digestions (*Hpa*II, *Hind*III, *Msp*I, *Bgl*II, and *Cfo*I) or double digestions (*Hpa*II/*Hind*II and *Msp*I/*Hind*III) performed on the DNA of the four patients and their parents displayed in each case expected band sizes (data not shown) except with the *Bgl*II restriction enzyme (Fig. 1). In families 1, 2, and 3 the patient and one parent displayed the expected 8.0-kb band with an additional 6.4 kb-band, and the other parent exhibited only the 8.0-kb band (Fig. 1b, d, f). In family 4, the patient exhibited the 8.0-kb band concomitantly with the 6.4-kb band, whereas the father and the mother respectively presented a single 6.4- and 8.0-kb band (Fig. 1h).

Taken together, these results showed that the *Bgl*II/*Cfo*I particular profiles observed in these families could not be the consequence of a microdeletion in the PW71 chromosomal region. The finding of heterozygote profiles in two fathers and one mother following *Bgl*II DNA digestion, combined with the fact that the father in family 4 displayed an homozygote profile, strongly supported the hypothesis of a restriction site polymorphism(s) and ruled out a particular methylation imprint. Using previously established restriction size fragments in the region [Dittrich et al., 1993; Zesch-nick et al., 1997] we provide a new map (Fig. 2) that demonstrates that the methylation process at the PW71 locus because of imprinting can provide the different observed profiles using *Bgl*II/*Cfo*I as the consequence of a unique *Bgl*II restriction polymorphism. To

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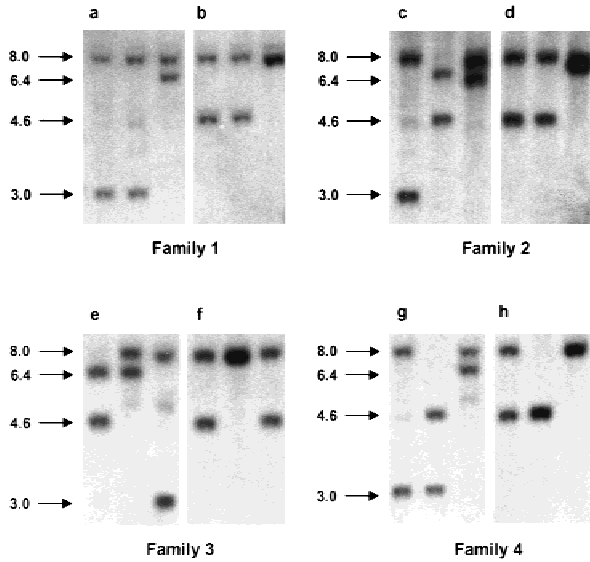


Fig. 1. Southern blot analysis with probe PW71B. Genomic DNA was digested with *Bgl*III + *Cfo*I (a, c, e, g) or *Bgl*III (b, d, f, h). From left to right: Patient, father, mother. In families 1, 2, and 3, patient was suspected of AS; in family 4, patient was suspected of PWS. DNA was prepared from whole blood.

confirm this hypothesis, 95 control DNAs were submitted to a *Bgl*III digestion followed by a PW71B Southern blot. Because two of the four patients were of North-African origin, 63 individuals from Algeria and Morocco and 32 from Europe were chosen. We found that four of individuals (1 female, 3 males), all of Algerian and Moroccan descent, displayed an heterozygote profile

and therefore exhibited the *Bgl*III polymorphism (data not shown).

Consequently, the three new and unusual Southern blot profiles that we found in four patients and one of their parents, with the PW71B probe and the restricting enzymes *Cfo*I and *Bgl*III, all derive from the same restriction polymorphism at a *Bgl*III enzyme recognition site. The multiplicity of the profiles is caused by parent-of-origin DNA methylation depending on the 15q11-q13 chromosomal region. Hence, we demonstrate that patients displaying such atypical profiles indeed do not present the 15q11-q13 DNA methylation alteration classically documented in PWS- and most AS-affected patients, but a normal biparental DNA methylation. Our data also suggest that this restriction polymorphism is likely to have a North-African origin. The report of these unusual profiles may therefore constitute a useful tool to avoid a potential pitfall in PWS and AS molecular diagnosis in North-African or Mediterranean patients. In addition, such an observation tips the balance of deciding whether to use PW71B or SNRPN methylation-sensitive probes further towards the use of SNRPN, as all the patients displayed a normal biparental profile with this probe. New methylation analysis techniques, based on sodium bisulfite treatment of DNA followed by polymerase chain reaction amplification, have been recently described for the diagnosis of PWS and AS [Kosaki et al., 1997; Kubota et al., 1997]. Because they evaluate the CpG island methylation status of the *SNRPN* gene, such procedures would therefore avoid the potential pitfall of PWS and AS molecular diagnosis because of the *Bgl*III polymorphism.

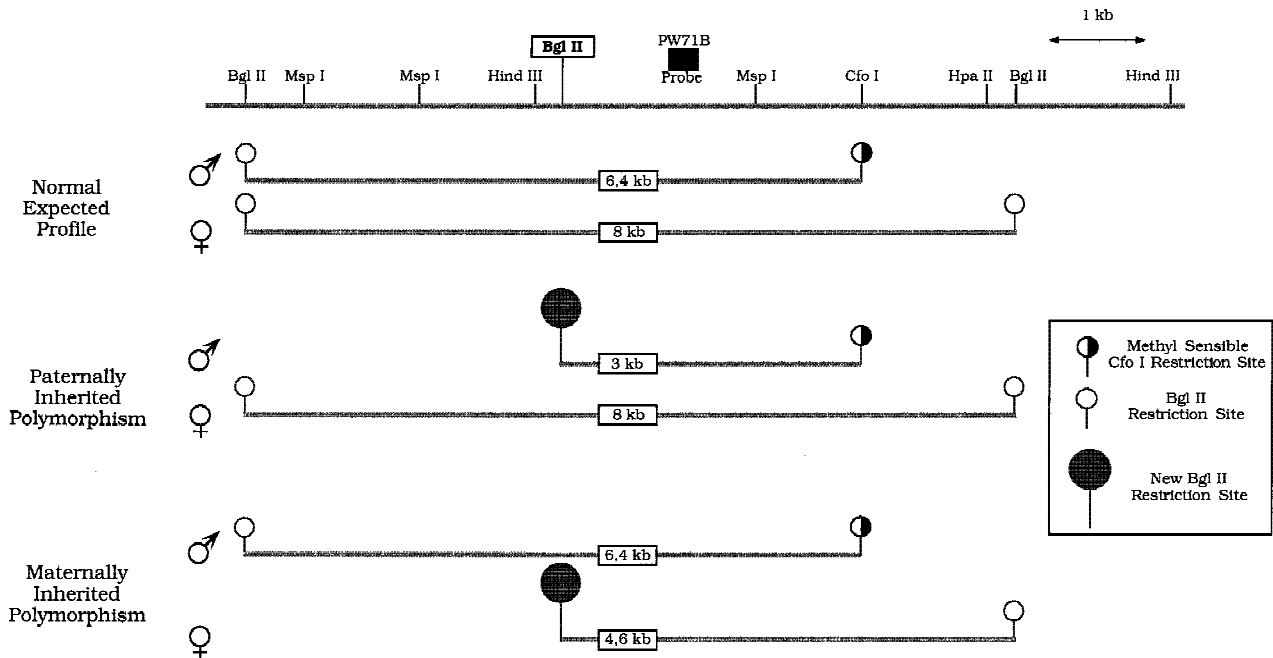


Fig. 2. Restriction map of the PWS/AS region on human chromosome 15q11-q13 containing PW71 and including the *Bgl*III polymorphism reported in this study [Dittrich et al., 1993; Zeschnigk et al., 1997].

## NOTE ADDED IN PROOF

As two additional patients of African descent were found to display the *Bgl*II polymorphism, the mutation origin may therefore be extended to other parts of the African continent.

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