A 28-kb Deletion Spanning *D15S63* (PW71) in Five Families: A Rare Neutral Variant?

Karin Buiting,[1] Bärbel Dittrich,[1] Bernd Dworniczak,[2] Israela Lerer,[3] Dvorah Abeliovich,[3] Sally Cottrell,[4] I. Karen Temple,[5] John F. Harvey,[6] Christina Lich,[1] Stephanie Groß,[1] and Bernhard Horsthemke[1]

[1]Institut für Humangenetik, Universitätsklinikum Essen, Essen; [2]Institut für Humangenetik, Westfälische Wilhelms-Universität Münster, Münster; [3]Hadassah University Hospital Kiryat Hadassah, Jerusalem; [4]The Institute of Child Health, University of London, London; [5]Wessex Clinical Genetics Service, Southampton University Hospitals NHS Trust, Southampton, and [6]Wessex Regional Genetics Laboratory, Salisbury Health Care NHS Trust, Salisbury

Summary

Methylation analysis with probe PW71 (*D15S63*) is an established procedure to test patients suspected of having Prader-Willi syndrome or Angelman syndrome. Using this test, we have identified a 28-kb deletion spanning *D15S63* in five independent families. Sequence analysis revealed identical breakpoints in all the families. The haplotype data are compatible with a common ancestral origin of the deletion in at least two families. The deletion was not found in 1,000 unrelated controls. Although the deletion maps within the imprinting-center region, neither maternal nor paternal inheritance of the deletion appears to affect imprinting in proximal 15q. We conclude that the deletion is a rare neutral variant that can lead to false-positive results in the PW71-methylation test.

Introduction

Prader-Willi syndrome (PWS [MIM 176270]) and Angelman syndrome (AS [MIM 105830]) are distinct neurogenetic disorders that are caused by a deficiency of imprinted gene expression from the paternal or maternal chromosome 15q11–q13, respectively (for review, see Nicholls et al. 1998). At the molecular level, the paternal and maternal copies of the imprinted PWS/AS region can be distinguished by DNA methylation, DNA replication, and gene expression. Parent-of-origin–specific DNA methylation can be used to confirm the clinical diagnosis of patients with either PWS and AS who have a deletion of 15q11–q13, uniparental disomy, or an imprinting defect. Imprinting defects can result from an imprinting-center (IC) mutation (Buiting et al. 1995; Saitoh et al. 1996; Ohta et al. 1999*a*, 1999*b*) or can occur spontaneously (Buiting et al. 1998; Ohta et al. 1999*b*). For methylation analysis, genomic DNA is digested with methylation-sensitive restriction enzymes and is analyzed by Southern blot hybridization or PCR (Dittrich et al. 1992, 1996*a*; Sutcliffe et al. 1994; Glenn et al. 1996; Chotai and Payne 1998) or is subjected to bisulfite treatment and methylation-specific PCR (Kosaki et al. 1997; Kubota et al. 1997; Zeschnigk et al. 1997). On the basis of extensive data obtained by methylation analysis of PWS and AS patients with PW71 (*D15S63*) and *SNRPN* probes, the ASHG/ACMG Test and Technology Transfer Committee (1996) has established this analysis as a scientifically and clinically valid test. Using probe PW71, we have identified a rare, 28-kb deletion that can confound routine diagnostic testing of patients.

Received July 12, 1999; accepted September 3, 1999; electronically published October 21, 1999.

Address for correspondence and reprints: Dr. B. Horsthemke, Institut für Humangenetik, Universitätsklinkum Essen, Hufelandstrasse 55, 45122 Essen, Germany. E-mail: b.horsthemke@uni-essen.de

0002-9297/1999/16506-0014$02.00

Patients, Material, and Methods

Patients

Family B.—The index patient had a birth weight of 2.3 kg. Microcephaly was noted at birth, and severe developmental delay was clear from the early neonatal period. She developed no speech. Epilepsy started at age 8 years. At age 26 years, her height and head circumference were below the 3d centiles. Her dysmorphic appearance included deep-set eyes and a prominent midface and upper jaw, but no prominence of the lower jaw. She had normal secondary sexual characteristics. She walked with a wide-based gait but did not have jerky movements or episodes of abnormal laughter.

Family 221.—The index patient is a 5-year-old boy. He was born with no problems after normal pregnancy; birth weight was 3 kg. He has mental developmental

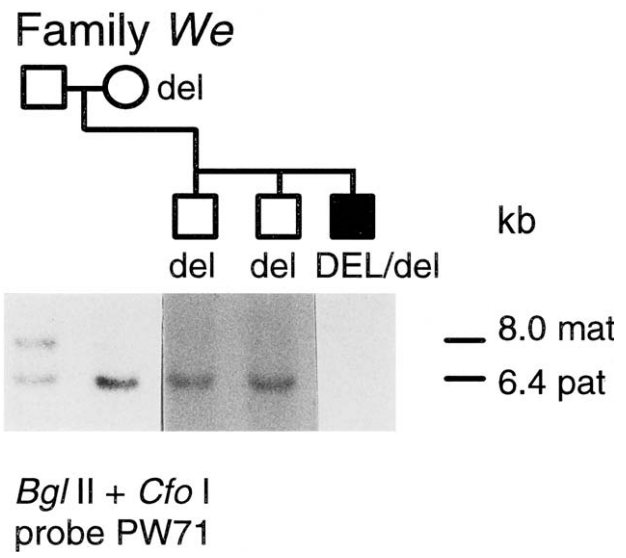


Figure 1 PW71-methylation analysis in the family of patient We. Normal individuals typically have a methylated maternal band of 8 kb and an unmethylated paternal band of 6.4 kb. The patient lacks both bands, whereas his healthy brothers and their mother lack the maternal band. del = 28-kb deletion; DEL = large deletion of 15q11–q13.

delay and problems in communication and language, and there are bursts of laughter. He also suffered from infantile spasm and stereotypic movements. He has a normal head circumference, a normal computed-tomography scan, and a normal magnetic resonance imaging of the brain.

Family 63.—The index patient is the sister of a girl with PWS. From the first session of genetic counseling, it was only noted that she has mental retardation (MR) and infantile spasm. Since the parents were not interested in further studies, a reexamination of this patient was not possible.

Family We.—The index patient is a 14-year-old boy with PWS. He has several clinical features typical of PWS, such as neonatal muscular hypotonia, feeding problems in the first period after birth, kryptorchidism, and hypopigmentation but lacks overt obesity.

Family P.—The 7-year-old index patient was first suspected of having AS. This diagnosis could not be confirmed by further clinical examinations. The patient has MR, features of autism, and delayed development of speech.

Subcloning of the Junction-Fragment and Deletion-Breakpoint Regions

An aliquot of genomic DNA (50 μg) from individual HW was completely digested with *Hin*dIII and was size fractionated on a 10%–40% sucrose gradient. The fraction containing the 15-kb *Hin*dIII junction fragment was identified through Southern blot hybridization with probe 71.19.12HR. An aliquot of this fraction (400 ng of DNA) and 1 μg of *Hin*dIII+*Xba*I-digested Lambda Dash II DNA (Stratagene) were ligated overnight with T4 DNA ligase. The ligation products were packaged in vitro with the Gigapack II Gold packaging extract (Stratagene), to yield a total of 4.5×10^4 plaque-forming units on host XL1-Blue MRA (P2). Approximately 400,000 plaques were screened with probe 71.19.12HR, and three positive plaques were obtained. After one round of plaque purification, phage DNA was isolated by standard methods. One phage (λHW42) was positive for both probes—71.19.12HR (centromere) and YR9AB (telomere)—that flank the breakpoint and that therefore are likely to contain the 15-kb *Hin*dIII junction fragment. Both 0.9- and 0.8-kb *Bgl*II fragments of the phage insert, termed "HWAF1" and "HWAF2," respectively, were subcloned into pT7T3-18U vector DNA (Pharmacia) and were sequenced.

The 2.5-kb *Bam*HI/*Bgl*II fragment of clone L48.19I was termed "HWNFT" and represents the normal telomeric genomic breakpoint region. The 3.6-kb *Bgl*II/*Pst*I fragment of clone 71.19.13 was termed "HWNFC" and represents the normal centromeric genomic breakpoint region. HWNFT was subcloned into *Bam*HI-digested pT7T3-18U vector DNA, and HWNFC was subcloned into *Bam*HI/*Pst*I-digested pT7T3-18U vector DNA. Both clones were partially sequenced.

DNA Sequence Analysis

Plasmid DNA was isolated from 1.5-ml bacterial cultures by use of the QIAscreen procedure (Diagen) and was dissolved in 20 μl of water. PCR products were purified with Microcon-100 microconcentrators (Amicon). DNA was sequenced with fluorescence-tagged dideoxynucleotides and the *Taq* cycle sequencing procedure (Applied Biosystems), with use of one of the following primers: M13/pUC reverse and sequencing primer, primer HWs 5′-AGT ATG ATT CAG AAG GCT GG-3′ (annealing temperature, 58°C) and primer HWp 5′-TCT ATG GAT TGC AAA AGT TG-3′ (annealing temperature, 54°C). Sequencing reactions were analyzed on an Applied Biosystems Automatic DNA Sequencer, model 377.

PCR Analysis of the Deletion-Junction Fragment

PCR conditions were as follows: 50 ng genomic DNA were added to a 50-μl reaction volume containing 200 μM each dNTP, 1 μM each primer, 2.5 U Ampli*Taq*, 1.5 mM $MgCl_2$, 50 mM KCl, 10 mM Tris-HCl pH 8.3, and 0.1% gelatin. PCR was performed in a GeneAmp 9600 thermal cycler (PE Biosystems), with an initial denatur-

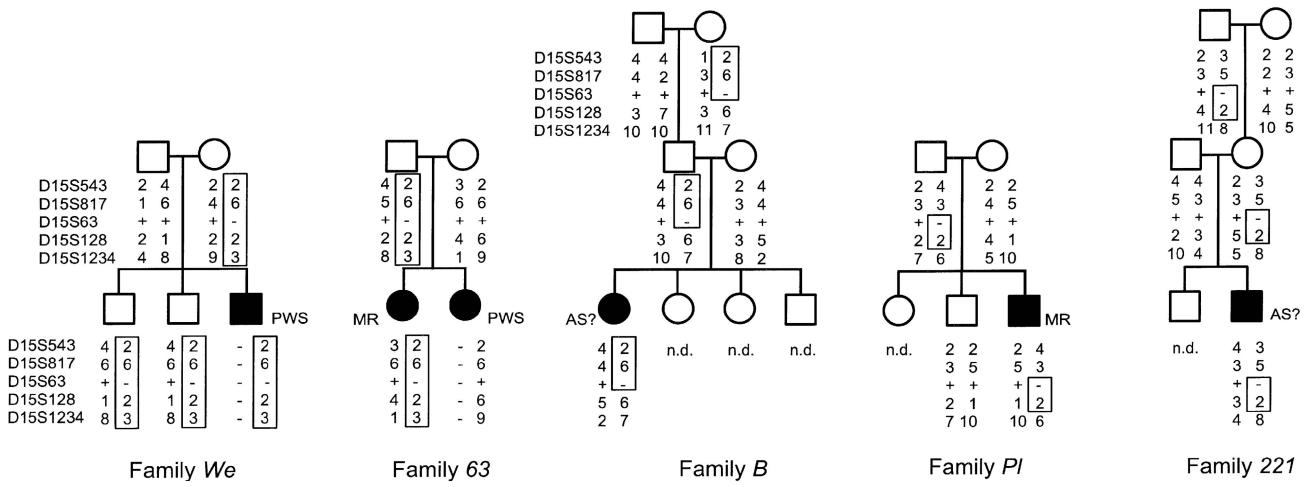


Figure 2 Haplotype analysis with chromosome 15–specific microsatellite markers. The loci *D15S543*, *D15S817*, *D15S128*, and *D15S1234* (centromere to telomere) map within the PWS/AS region. Common alleles are boxed.

ation for 5 min at 94°C. Thirty-five cycles were run, with denaturation for 15 s at 94°C, annealing for 15 s at a primer-specific temperature, and elongation for 30 s at 72°C. The final elongation was for 5 min at 72°C. The annealing temperature for primers HWs and HWp was 54°C.

For the screening of unrelated individuals, DNA samples from 10 individuals each were pooled. Aliquots of these pools (250 ng DNA) were used for PCR with primers HWs and HWp. As a positive control, a primer pair—OS1-860u (5′-CAG CAG GAG AAT ATG CAG-3′) and OS1-979l (5′-TGG CAG TAA GTG TTT ATC TGG-3′)—specific for a locus on chromosome 8q was included in the PCR. By including DNA from the mother of patient We in one pool, we established that the screening assay was sensitive enough to detect 1 deletion carrier among 10 individuals.

DNA Methylation and Southern Blot Analysis

Genomic DNA was purified from whole blood, according to standard methods. Aliquots of the DNA (2 μg) were digested with the appropriate restriction enzyme. Fragments were separated by gel electrophoresis and were transferred to Biodyne A nylon membranes (PALL). Blots were hybridized as described elsewhere (Buiting et al. 1998).

Genotyping

Microsatellite analysis was performed with the following fluorochromed markers from proximal 15q11–q13: *D15S543*, *D15S817*, *D15S128*, and *D15S1234*. Amplification products were analyzed with the A 310 Genetic Analyzer (Applied Biosystems) and GENOTYPER software.

Probes

To characterize the deletions by Southern blot analysis, the following probes were used: 71.19.12HR (Saitoh et al. 1996), YR9AB (Dittrich et al. 1994), and kb19, a 500-bp genomic fragment that maps ~8 kb proximal to YR9AB (K. Buiting, unpublished data). Methylation analysis was performed with probe PW71B, for the *D15S63* locus, and with kb17 (Buiting et al. 1998), for the *SNRPN* exon 1 region.

Results

In the course of routine diagnostic testing, we found a German patient (We) with PWS and an apparently homozygous deletion for PW71 (*D15S63*; fig. 1). Repeated Southern blot hybridizations with PW71 never gave a signal with the patient's DNA, whereas control probes for other loci did (data not shown), thus demonstrating the integrity of the DNA sample. Interestingly, the two healthy brothers and their healthy mother lacked a maternal band—that is, showed an "AS" pattern. Microsatellite studies (fig. 2) and quantitative Southern blot hybridizations with PW71 (data not shown) revealed that the patient has a common large deletion on his paternal chromosome and a small deletion spanning the PW71 locus on his maternal chromosome. The methylation analysis suggested that the small deletion was also present in the two healthy sibs and in their mother.

Knowing about this deletion, we investigated other families with an unusual PW71 result. As shown in table 1, we identified four individuals with an abnormal PW71-methylation pattern but with a normal *SNRPN*-methylation pattern. This group of individuals included two patients with unspecific MR (63 and Pl) and one

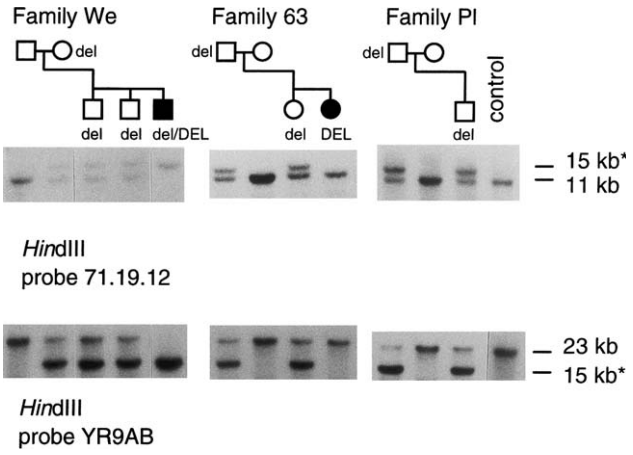


Figure 3 Identification of the proximal and the distal deletion breakpoints by Southern blot analysis. The junction fragments are indicated by an asterisk (*).

patient suspected of having AS (B), who, surprisingly, lacked the paternal PW71 band—that is, showed a "PWS" pattern. Patient 221, who was suspected of having AS, did show an "AS" methylation pattern with PW71, but subsequent molecular work-up of the patient revealed a normal *SNRPN*-methylation pattern. We reasoned that the findings in these four patients may also be explained by a PW71 deletion and set out to investigate the deletion at the nucleotide level.

Characterization of the Deletion

First, we performed Southern blot hybridizations with probes flanking PW71. In *Hin*dIII-digested DNA from patients We, 63, and Pl, we detected a putative deletion-junction fragment of 15 kb, with probes 71.19.12 HR and YR9AB (see fig. 3; patients B and 221 had not yet been identified at this stage of our study).

Refined restriction analysis of DNA from the eldest brother of patient We (HW) revealed that the proximal deletion breakpoint maps inside a 3.6-kb *Bgl*II/*Pst*I fragment (HWNFC), between the anonymous markers 71.19.12HR and PW71, and that the distal breakpoint lies in a 2.5-kb *Bam*HI/*Bgl*II fragment (HWNFT), between the anonymous markers kb19 (cen) and YR9AB (tel). Interestingly, the distal breakpoint of the deletion maps within a previously reported 1.5-kb insertion/deletion element, which represents a neutral DNA polymorphism (Dittrich et al. 1994; Gabriel et al. 1994). On the basis of these data, the deletion was estimated to span ~28 kb (fig. 4).

To analyze the deletion at the nucleotide level, we subcloned the 15-kb *Hin*dIII junction fragment from HW and identified one positive phage clone (λHW42). Since a *Bgl*II site flanks HWNFC (3.6 kb) at its centromeric end and flanks HWNFT (2.5 kb) at its telomeric end, we subcloned the two *Bgl*II fragments of λHW42 that were <6.1 kb (HWAF1, 0.9 kb, and HWAF2, 0.8 kb).

Sequence comparisons of clones HWAF1, HWAF2, HWNFT, and HWNFC revealed that HWAF2 represents the junction fragment. HWAF2 contains—from centromere to telomere—a 50-bp single-copy sequence, a partial *Alu* sequence, a complete *Alu* sequence, two partial *Alu* sequences and a 216-bp single-copy sequence. Sequencing of HWNFC, HWNFT, and HWAF2 with specific primers that recognize the centromeric (primer HWs) and telomeric (primer HWp) single-copy sequence of HWAF2 revealed that all three fragments are identical over an 18-bp interval (see fig. 4). These data suggest that a recombination event that led to the deletion of 28 kb in individual HW has occurred in an 18-bp interval that starts at nucleotide position 205 of HWAF2 and that is located in the complete *Alu*- sequence.

To determine whether the junction fragments in all five families are identical, we performed PCR analysis using genomic DNA and specific primers flanking the breakpoints (primers HWs and HWp, see above). As shown in figure 5, a 612-bp fragment was amplified in all individuals previously identified as carrying the deletion. Sequence analysis of the fragments revealed complete sequence identity in all cases, and therefore the five families have the same 28-kb deletion.

Frequency of the Deletion

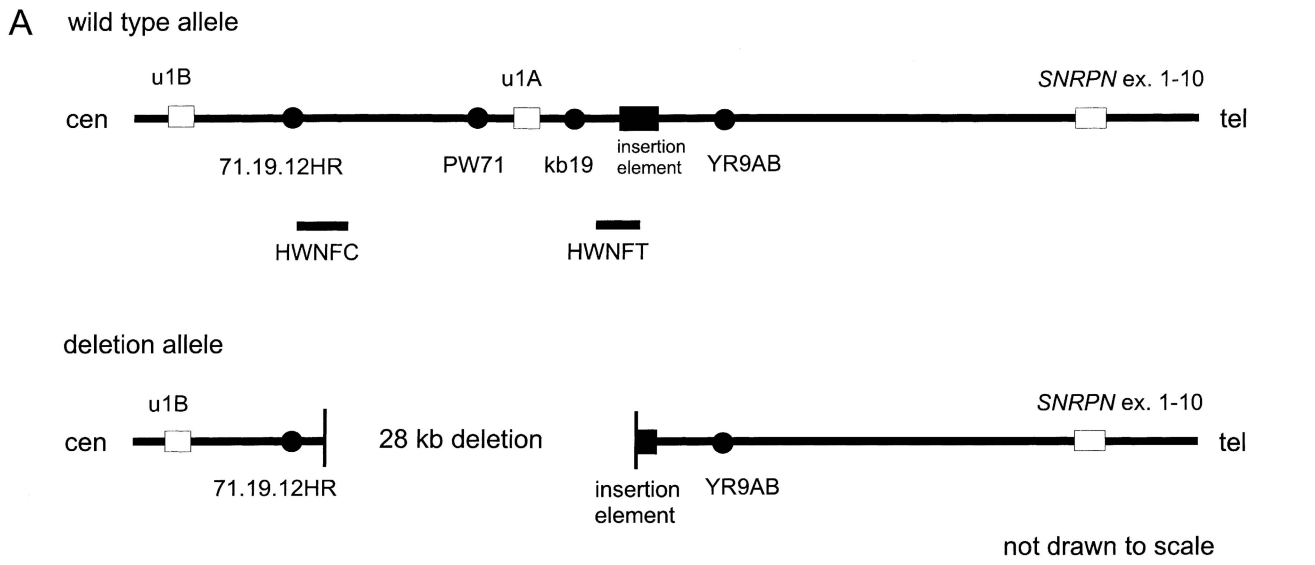
To determine the frequency of the PW71 deletion, 1,000 unrelated individuals were investigated. Fifty-seven individuals were studied, by Southern blot analysis, for the presence of the 15-kb *Hin*dIII junction fragment. The other individuals (943) were screened by PCR with primers HWs and HWp. To check for the integrity of the genomic DNA, control primers for a chromosome 8 locus (O. Schmidt et al., personal communication) were included in the PCR. None of the investigated individuals had the 28-kb deletion.

Table 1

Patients with Unusual PW71 Results

Patient	Clinical Diagnosis[a]	Country	Methylation Pattern[b]	
			PW71	*SNRPN*
We	PWS	Germany	Homozygous deletion	"PWS"
63	MR	Israel	"PWS"	Normal
Pl	MR	Germany	"PWS"	Normal
B	AS?	England	"PWS"	Normal
221	AS?	Israel	"AS"	Normal

[a] ? = Clinical diagnosis uncertain.
[b] "PWS" = lack of paternal band; "AS" = lack of maternal band.



B

	cen		tel
HWNFC	GGCCGGGTGCAGTGGCTCAT	GCCTGTAATCCCAGCACT	TTGGGAGGCTGAGGCAGGCG
junction fragment	GGCCGGGTGCAGTGGCTCAT	GCCTGTAATCCCAGCACT	GTGGGAGGCCAAGGCGGGCG
HWNFT	GGCTGGGCGCAGTGGCTCAC	GCCTGTAATCCCAGCACT	GTGGGAGGCCAAGGCGGGCG

Figure 4 *A,* Genomic map of the PW71/SNRPN region, showing the normal allele and the deletion allele. *B,* Partial sequence of HWNFC and HWNFT fragments, which contain the proximal and distal deletion-breakpoint region, respectively, and the deletion junction fragment. The recombination has occurred between two *Alu* repeats within a stretch of 18 bp. Divergent nucleotides are underlined.

Haplotype Analysis

To determine whether the deletion occurred on a common ancestral haplotype, we performed microsatellite analysis with markers from the PWS/AS region (fig. 2). Interestingly, in families We and 63, the affected chromosomes share alleles at four loci flanking the deletion (*D15S543* and *D15S817* at the proximal side and *D15S128* and *D15S1234* at the distal side). The *D15S543/D15S817* subhaplotype was also found in family B, and, in families Pl and 221, the same *D15S128* allele that was seen in We and 63 was found.

Discussion

Here we have described the finding of a 28-kb deletion spanning the PW71 locus on chromosome 15 in five different families. Partial sequence analysis revealed that the deletion arose by *Alu-Alu* recombination between an *Alu* repeat inside a polymorphic insertion/deletion element and another *Alu*-repeat 28-kb centromeric. The identity of the deletion breakpoints suggests that it represents a rare neutral variant. The frequency of this variant appears to be <.001, at least in Germany, North America, and Australia. This estimate is based on the following findings. First, there are three extensive studies describing a comparison between the PW71- and *SNRPN*-methylation patterns (Kubota et al. 1996; Buchholz et al. 1997; Zeschnigk et al. 1997). No discrepancy was observed between the methylation patterns at the two loci in the 690 patients with either PWS or AS and normal controls tested in these studies, suggesting that none of the individuals tested carried the deletion. In PWS and AS patients with uniparental heterodisomy, the methylation pattern may mask such a deletion, but these patients represent the minority of the investigated individuals. Second, we did not find the deletion in 1,000 unrelated individuals.

The haplotype data are compatible with a common ancestral origin of the deletion, in at least two families. In view of the large genetic distance between the available markers in the PWS/AS region, however, it cannot be excluded that the other three families, who have at least one allele in common with the other two families, are also related to this putative common ancestor. Although two families are from Israel and the mother of patient We has Jewish ancestors, the data are not sufficient to determine whether the deletion is of Jewish origin.

Although in family 221 the inheritance pattern of the deletion appears to be like that of an imprinting mu-

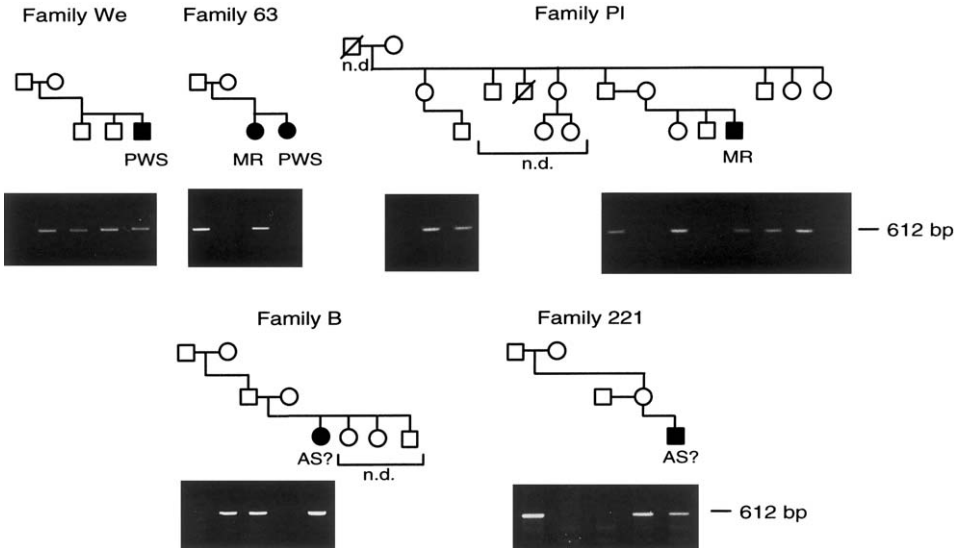


Figure 5 Deletion junction PCR. Individuals carrying the 28-kb deletion are positive for a 612-bp PCR product.

tation, and although the deletion affects one of the alternative start exons of the putative IC transcripts (exon u1A, previously designated "BD1A" [Dittrich et al. 1996*b*; Färber et al. 1999]), there is no evidence that the deletion impairs imprinting in this region. (1) The 28-kb deletion does not overlap with the smallest regions of deletion overlap in AS (Ohta et al. 1999*a*) and PWS (Ohta et al. 1999*b*), which map ~50 and ~85 kb more telomeric, respectively. (2) The identification of the deletion in patients suspected of having either PWS or AS is most likely due to an ascertainment bias—other patients would not have been tested for methylation at this locus—and does not imply a causal relationship between the deletion and the disease. There are six deletion carriers with a normal phenotype who inherited the deletion from the mother and eight deletion carriers who inherited the deletion from the father. It is possible that the IC transcripts are not necessary for imprinting or that expression from the remaining start exon (u1B, previously designated "BD1B"), which is not affected in these individuals, is sufficient for the proposed role of the IC transcripts in the imprinting process.

Although the PW71 deletion represents a very rare variant, our findings have important implications for routine diagnostic testing of patients suspected of having either PWS or AS. The deletion may lead to false-positive PW71-methylation results, and it is recommended that diagnostic laboratories that have obtained unexpected or unusual results test their samples for the presence of the deletion, by using the deletion-junction PCR assay described in this report. In contrast to the PW71 deletion, all deletions including the *SNRPN* promoter/exon 1 region appear to be associated with an imprinting defect (Sutcliffe et al. 1994; Buiting et al. 1995; Saitoh et al. 1996; Ohta et al. 1999*b*). Therefore, false-positive methylation results at this locus are less likely, unless there are technical problems with either the *Not*I digest or the methylation-specific PCR. Although we now prefer the methylation-specific PCR analysis of *SNRPN*, it may be necessary to test both PW71 and *SNRPN*. This is because we have recently identified two patients with a normal *SNRPN*-methylation pattern but a truly abnormal methylation pattern at PW71 and other differentially methylated sites in the region (K. Buiting and B. Horsthemke, unpublished data).

Acknowledgments

We thank Britta Hitschfeld for expert technical assistance; Dr. Olaf Schmidt for kindly providing primers from chromosome 8q24.1; Drs. Sue Malcolm, Marcus Pembrey, Gabriele Gillessen-Kaesbach, J. Horst, and B. Wittwer and R. Exeler for contribution to this work. Part of this work was supported by the Deutsche Forschungsgemeinschaft.

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 AS [MIM 105830] and PWS [MIM 176270]).

References

ASHG/ACMG Test and Technology Transfer Committee (1996) Diagnostic testing for Prader-Willi and Angelman syndromes: report of the ASHG/ACMG test and technology transfer committee Am J Hum Genet 58:1085–1088

Buchholz T, Jackson J, Smith A (1997) Methylation analysis at three different loci within the imprinted region of chromosome 15q11-13. Am J Med Genet 72:117–119

Buiting K, Dittrich B, Groß S, Lich C, Färber C, Buchholz T, Smith E, et al (1998) Sporadic imprinting defects in Prader-Willi syndrome and Angelman syndrome: implications for imprint-switch models, genetic counseling, and prenatal diagnosis. Am J Hum Genet 63:170–180

Buiting K, Saitoh S, Groß S, Dittrich B, Schwartz S, Nicholls R, Horsthemke B (1995) Inherited microdeletions in the Angelman and Prader-Willi syndromes define an imprinting center on human chromosome 15. Nat Genet 9:395–400

Chotai KA, Payne SJ (1998) A rapid, PCR based test for differential molecular diagnosis of Prader-Willi and Angelman syndromes. J Med Genet 35:472–475

Dittrich B, Buiting K, Groß S, Horsthemke B (1994) An insertion/deletion polymorphism at the D15S63 locus in the critical Prader-Willi syndrome region in 15q11-13. Hum Genet 94:583–584

Dittrich B, Buiting K, Horsthemke B (1996*a*) PW71 methylation test for Prader-Willi and Angelman syndromes. Am J Med Genet 61:196–197

Dittrich B, Buiting K, Korn B, Rickard S, Buxton J, Saitoh S, Nicholls RD, et al (1996*b*) Imprint switching on human chromosome 15 may involve alternative transcripts of the SNRPN gene. Nat Genet 14:163–170

Dittrich B, Robinson W, Knoblauch H, Buiting K, Schmidt K, Gillessen-Kaesbach G, Horsthemke B (1992) Molecular diagnosis of the Prader-Willi and Angelman syndromes by detection of parent-of-origin specific DNA methylation in 15q11-13. Hum Genet 90:313–315

Färber C, Dittrich B, Buiting K, Horsthemke B (1999) The chromosome 15 imprinting center (IC) region has undergone multiple duplication events and contains an upstream exon of SNRPN that is deleted in all Angelman syndrome patients with an IC microdeletion. Hum Mol Genet 8:337–343

Gabriel J, Gottlieb W, Garcia A, Rogan PK, Saitoh S, Nicholls RD (1994) A common insertion/deletion polymorphism in the Prader-Willi syndrome minimal critical region. Hum Mol Genet 10:1912

Glenn CC, Saitoh S, Jong MTC, Filbrandt MM, Surti U, Driscoll DJ, Nicholls RD (1996) Gene structure, DNA methylation and imprinted expression of the human *SNRPN* gene. Am J Hum Genet 58:335–346

Kosaki K, McGiniss MJ, Veraksa AN, McGiniss WJ, Jones KL (1997) Prader-Willi and Angelman syndromes: diagnosis with a bisulfite-treated methylation-specific PCR method. Am J Med Genet 73:308–313

Kubota T, Das S, Christian SL, Baylin SB, Herman JG, Ledbetter DH (1997) Methylation specific PCR simplifies imprinting analysis. Nat Genet 16:16–17

Kubota T, Sutcliffe JS, Aradya S, Gillessen-Kaesbach G, Christian SL, Horsthemke B, Beaudet AL, et al (1996) Validation studies of *SNRPN* methylation as a diagnostic test for Prader-Willi syndrome. Am J Med Genet 66:77–80

Nicholls RD, Saitoh S, Horsthemke B (1998) Imprinting in Prader-Willi and Angelman syndromes. Trends Genet 14: 194–199

Ohta T, Buiting K, Kokkonen H, McCandless S, Heeger S, Leisti H, Driscoll DJ, et al (1999*a*) Molecular mechanism of Angelman syndome in two large families involves an imprinting mutation. Am J Hum Genet 64:385–396

Ohta T, Gray TA, Rogan PK, Buiting K, Gabriel JM, Saitoh S, Muralidhar B, et al (1999*b*) Imprinting mutation mechanisms in Prader-Willi syndrome. Am J Hum Genet 64: 397–413

Saitoh S, Buiting K, Rogan PK, Buxton JL, Driscoll DJ, Arnemann J, König R, et al (1996) Minimal definition of the imprinting center and fixation of a chromosome 15q11-q13 epigenotype by imprinting mutations. Proc Natl Acad Sci USA 93:7811–7815

Sutcliffe JS, Nakao M, Christian S, Örstavik KH, Tommerup N, Ledbetter DH, Beaudet AL (1994) Deletions of a differentially methylated CpG island at the *SNRPN* gene define a putative imprinting control region. Nat Genet 8:52–58

Zeschnigk M, Lich C, Buiting K, Horsthemke B, Dörfler W (1997) A single-tube PCR test for the diagnosis of Angelman and Prader-Willi syndrome based on allelic methylation differences at the *SNRPN* locus. Eur J Hum Genet 5:94–98