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Submicroscopic Deletion in Cousins With Prader-Willi Syndrome Causes a Grandmatrilineal Inheritance Pattern: Effects of Imprinting

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The Prader-Willi syndrome (PWS) critical region on 15q11–q13 is subject to imprinting. PWS becomes apparent when genes on the *paternally* inherited chromosome are not expressed. Familial PWS is rare. We report on a family in which a male and a female paternal first cousin both have PWS with cytogenetically normal karyotypes. Fluorescence in situ hybridization (FISH) analysis shows a submicroscopic deletion of SNRPN, but not the closely associated loci D15S10, D15S11, D15S63, and GABRB3. The cousins' fathers and two paternal aunts have the same deletion and are clinically normal. The grandmother of the cousins is deceased and not available for study, and their grandfather is not deleted for SNRPN. DNA methylation analysis of D15S63 is consistent with an abnormality of the imprinting center associated with PWS. "Grandmatrilineal" inheritance occurs when a woman with deletion of an imprinted, paternally expressed gene is at risk of having affected grandchildren through her sons. In this case, PWS does not become evident as long as the deletion is passed through the matrilineal line. This represents a unique inheritance pattern due to imprinting. Am. J. Med. Genet. 92:19–24, 2000. © 2000 Wiley-Liss, Inc.

KEY WORDS: Prader-Willi syndrome; imprinting; imprinting center

INTRODUCTION

Prader-Willi syndrome (PWS) is characterized by neonatal hypotonia and feeding difficulty, followed by early childhood hyperphagia, obesity, hypogonadism, and developmental delay. The critical region for the Prader-Willi syndrome (PWS) on 15q11–q13 is subject to imprinting [Nicholls et al., 1989]. Clinical features of PWS become apparent when genes on the paternally inherited chromosome are not expressed [Butler and Palmer, 1983; Robinson et al., 1991]. Loss of an active PWS region is due to loss of a functional paternal copy, most frequently from a deletion of the paternal copy of the PWS critical region or paternal uniparental disomy. Mutations in an imprinting center that cause a failure to reset the imprint during meiosis have been described [Buiting et al., 1995]. The Angelman syndrome critical region also lies within 15q11–q13 but is distinct from that of PWS. This condition is due to loss of maternal material from this region [Knoll et al., 1989].

PWS is generally sporadic, but there are a few familial occurrences. Some of these recurrences were due to inheritance of an unbalanced translocation from a carrier father [Fernandez et al., 1987; Hasegawa et al., 1984; Hultén et al., 1991]. Several families have been reported in which sibs and their normal father all have submicroscopic deletions of 15q11–q13 that likely involve the imprinting center [Buiting et al., 1995; Ishikawa et al. 1996; Orstavik et al., 1992; Reis et al., 1994; Teshima et al., 1996]. We provide the first report of cousins with Prader-Willi syndrome with cytogenetically normal chromosomes. They and other relatives have a submicroscopic deletion involving 15q11–q13. We will discuss the implications for recurrence risk for other relatives and for genetic counseling. This family illustrates a unique pattern of inheritance due to effects of imprinting.

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CLINICAL REPORTS

Patient 1 (Propositus)

Patient 1 is a first cousin of patient 2; their fathers are brothers. He was born at 41 weeks of gestation weighing 3400 g. He had severe hypotonia in the neonatal period and required nasogastric feeding for the first 10 days of life. He also has cryptorchidism. On our examination at age 6 months, he had bitemporal narrowness, unilateral esotropia, hypotonia, and the foot length was less than the 3rd centile bilaterally.

Patient 2

Patient 2 (Fig. 1) is a girl born at 33 weeks gestation secondary to placental abruption weighing 1.5 kg (20th centile). She had profound neonatal hypotonia and required gavage feeding. She was also noted to have a hemivertebral body, fusion and hypoplasia of ribs, 13 ribs on the left, and fusion of cervical vertebrae 6 and 7. She had a high arched palate, epicanthus, and strabismus noted neonatally. Electroencephalogram, nerve conduction studies, and electromyogram were normal. A muscle biopsy was unrevealing. In early childhood, she developed hyperphagia and obesity. She had Blount disease at the age of 12 years. She also has diabetes mellitus and hypoventilation. At the age of 12 years 9 months, her height was less than the 5th centile (50th centile for 10.5 years) and her foot length was less than the 3rd centile. An evaluation at age 18 years showed an IQ of 49 on the Slosson Intelligence Test.

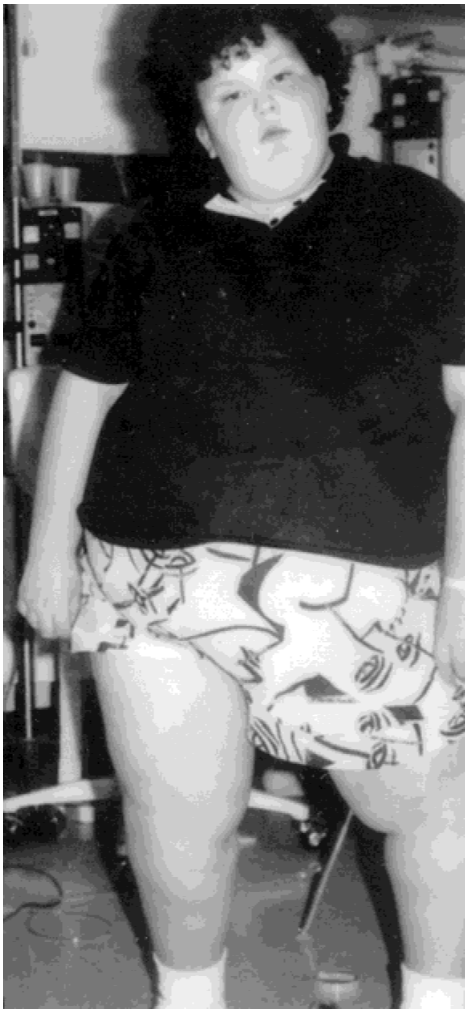

Fig 1. One of the affected cousins with PWS at age 16 years (Individual IV-2 in the pedigree in Fig. 2).

Both patients fulfill proposed diagnostic criteria for PWS [Holm et al., 1993]. Patient 1 had neonatal hypotonia, neonatal feeding problems, hypogonadism, developmental delay, molecular abnormality of PWS region, and small feet. Patient 2 had neonatal hypotonia, neonatal feeding problems, rapid weight gain, global developmental delay, characteristic face, hyperphagia, molecular abnormality of PWS region, short stature, small feet, and strabismus.

Family History

Patient 1 has 3 sibs who are healthy and developing normally (Fig. 2). Patient 2 had 1 sibling who was healthy with normal development until his death in a drowning accident. The patients' fathers also have no significant medical problems. The patients have 3 additional paternal uncles and 5 paternal aunts. The paternal grandmother died of emphysema. The paternal grandfather is healthy.

MATERIALS AND METHODS

Cytogenetic Studies

High-resolution karyotypes with G-banding were performed on the two affected cousins as well as their fathers.

Molecular Cytogenetic Analysis

Cosmid probes. Fluorescence in situ hybridization studies on metaphase spreads with probes for the markers SNRPN and D15S10 (Oncor Inc., Gaithersburg, MD) were performed on all relatives. A probe for the locus PML on 15q22 was used to identify the chromosome 15 homologues. Cosmids for the markers D15S11 and GABRB3 (Oncor Inc., Gaithersburg, MD) were also used for FISH in the 2 clinically affected cousins. FISH was carried out according to the manufacturer's instructions.

Fluorescence in situ hybridization probes. YAC clones were obtained from the Human Genome Project Bank. The clones 307A12, 326F6, and 457B7 were derived from the Centre d'Etude du Polymorphism Humaine (CEPH) [Albertsen et al., 1990]. Total yeast DNA as well as DNA from the 15 kb phage clone JP3 (D15S63) [Knoll et al., 1993] were labeled by nick translation with biotin-16 dUTP. Hybridization was performed as previously described [Knoll and Lichter, 1994].

DNA Methylation Analysis

PW71 methylation. Total genomic DNA was isolated from Patient 2 and her father (QIAGEN, Valencia, CA). The DNA was digested with *Hind*III and *Hpa*II, run on an agarose gel, transferred to a nylon

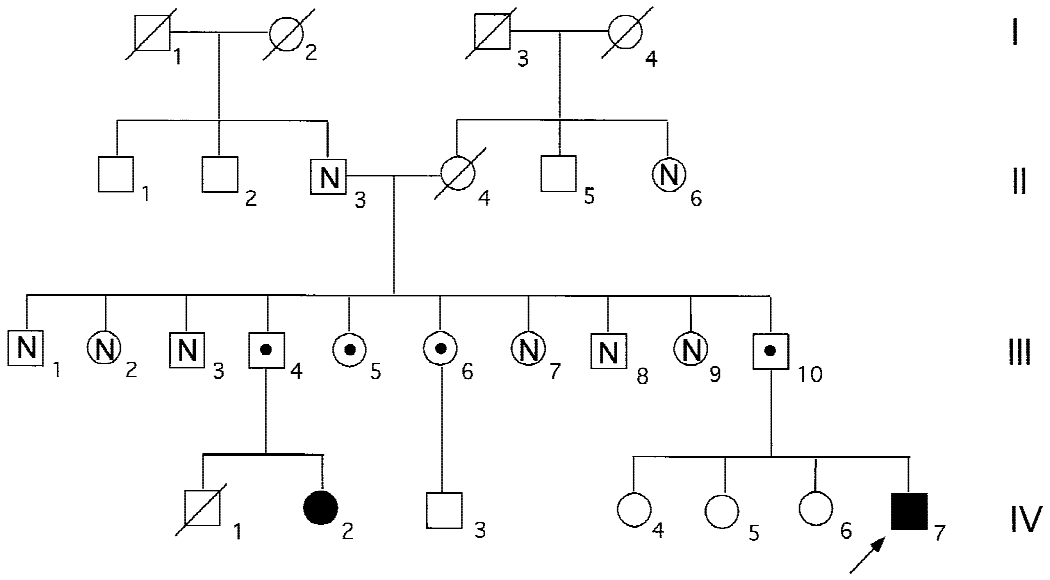

Fig. 2. Pedigree. The propositi are paternal first cousins; their fathers are brothers. The affected patient's fathers and two aunts carry the deletion but are clinically normal. Individual IV-1 died in an accident and had normal somatic and intellectual development to that point. ○, □: Deletion not tested, clinically normal. ●, ■: Prader-Willi syndrome. Ⓝ, [N]: No deletion, clinically normal. ⊙, ⊡: Carries deletion 46,XX/Y.ish del(15)(q11.2q11.2)(SNRPN–D15S10+ GABRB3+ D15S11+), clinically normal.

membrane, and hybridized with the PW71 probe (D15S63) by standard techniques [Buiting et al., 1995].

SNRPN methylation. Total genomic DNA was isolated from the father of patient 2 and two of her paternal aunts (III.2, III.5). Using previously described methods [Kubota et al., 1997], methylation-specific PCR was performed after treatment of the genomic DNA with the CpGenome (Oncor) DNA modification kit.

RESULTS

Molecular Cytogenetic Studies

Both affected individuals and their fathers all had cytogenetically normal karyotypes at the 550-band level. Molecular cytogenetic (FISH) studies on both patients revealed a deletion of SNRPN on one chromosome 15 homologue (Fig. 3A). D15S10 (Fig. 3B), GABRB3, and D15S11 were all present on both homologues. An identical pattern was detected in both of the patients' fathers (Fig. 2, individuals III-4, III-10), and in 2 of the fathers' 8 sibs (individuals III-5, III-6). SNRPN was not deleted in other relatives tested, including 3 paternal aunts, 2 paternal uncles, the paternal grandfather, and the paternal grandmother's sister. The paternal grandmother was deceased and no tissue was available for study.

To further delineate the deletion, selected YACs encompassing and flanking SNRPN [Mutirangura et al., 1993] were used as FISH probes (Fig. 4). These included YAC 307A12 (approximately 360 kb, containing locus D15S13), YAC 326F6 (335 kb, containing locus D15S63), and YAC 457B7 (320 kb, containing locus D15S174). As expected, YACs 307A12 and 326F6 were present on each chromosome 15 homologue in the index cases. YAC 457B4, that contains the SNRPN gene, was also present in two copies of strong intensity. In addition, a phage clone for D15S63 (JP3) was also used for FISH, and two copies were present in individuals with the SNRPN deletion. These data suggest that the deleted region is small compared to the 320 kb YAC.

DNA Methylation Studies

The PW71B probe detects DNA methylation at the D15S63 locus. When digested with *Hind*III and *Hpa*II, normal individuals have a maternally derived band of 6.0 kb, and a paternally derived band of 4.4 kb [Dittrich et al., 1992]. The affected Patient 2 showed only the maternal methylation pattern (Fig. 5). In combination with the FISH finding of two copies of D15S63 and deletion of one copy of SNRPN, these data are suggestive of a deletion affecting the imprinting center. Her father, who has the same FISH pattern as his daughter, showed both paternal and maternal methylation patterns.

To determine the origin of the deletion, we also performed methylation analysis of SNRPN on the father of Patient 2 (III.4), and two paternal aunts of this patient, one of whom had a deletion of SNRPN by FISH (III.5), and one of whom had two copies of SNRPN (III.2). Both the patient's father and the aunt with one copy of SNRPN showed only the presence of the paternally-imprinted 100 bp PCR product (data not shown). The aunt with two copies of SNRPN showed both the maternal (174 bp) and the paternal (100 bp) products. These results are consistent with the deletion being present in the patient's paternal grandmother.

DISCUSSION

We describe two paternal first cousins with PWS who have a submicroscopic deletion of 15q11–q13. Both children meet consensus diagnostic criteria for PWS [Holm et al., 1993]. This is the first report of cousins

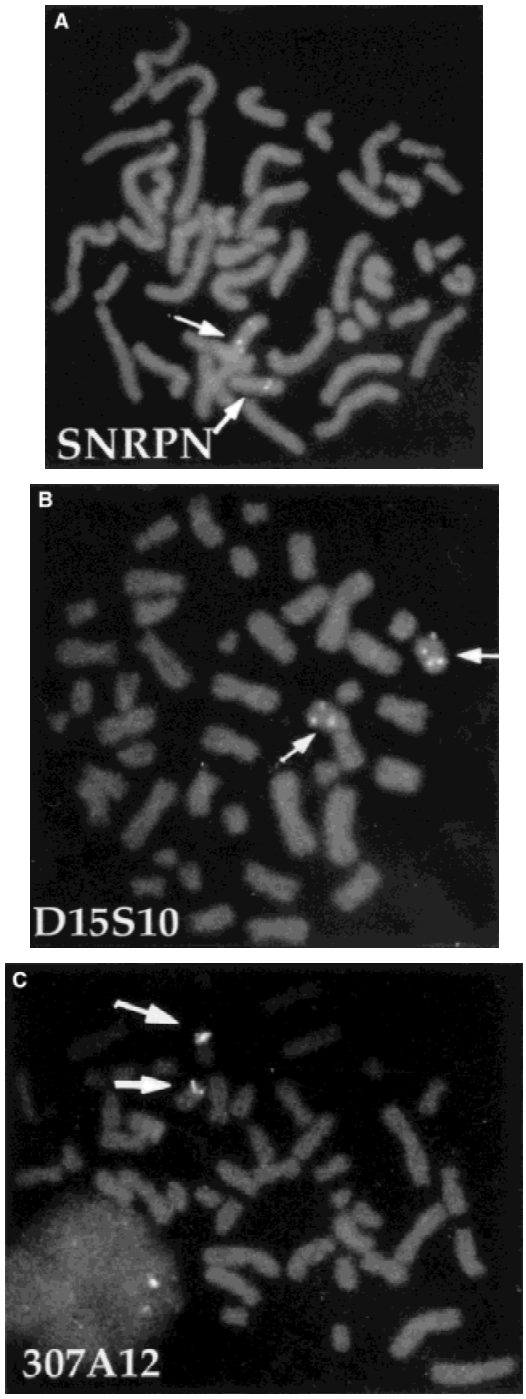

Fig. 3. FISH mapping of probes for 15q11–q13 on metaphases from individuals IV:2 or IV:7, who have PWS. (**A**) FISH of SNRPN with PML control. Only one copy of SNRPN is present. The PML marker indicates the two chromosome 15 homologues. (**B**) FISH of D15S10 with PML control. Both chromosome 15 homologues show hybridization to the probe for D15S10. (**C**) FISH of YAC 307A12 containing the marker D15S13. Both homologues show hybridization. Similar results were obtained with probes containing the markers D15S63 and D15S174.

affected with PWS who have cytogenetically normal chromosomes. In addition, the pedigree contains multiple clinically normal carriers who also carry the same submicroscopic deletion, including each of the patient's fathers and two paternal aunts (Fig. 3). The propositus's paternal grandfather did not carry the deletion, and the grandmother was not available for testing. We postulate that she also carries the deletion. The sibs of the propositus were not tested for the deletion because all of them had normal growth and development and were old enough so that clinical manifestations of PWS should have been apparent. Approximately 60% of individuals with PWS have a cytogenetically detectable deletion of 15q11–q13 [Ledbetter et al., 1987]. These deletions are almost always de novo and are generally 4 megabases in size. Commonly deleted loci include SNRPN, D15S10, GABRB3, and D15S11. Based on the FISH studies, the size of the deletion in the patients in this report is much smaller than the common deletion, as only the clone for SNRPN is deleted.

There have been several previous reports of familial recurrence of PWS. Some instances were due to unbalanced translocations involving chromosome 15 in sibs [Fernandez et al., 1987; Hultén et al., 1991]. Cousins inheriting an unbalanced translocation have also been reported [Hasegawa et al., 1984]. Although both were diagnosed with PWS, one individual inherited the abnormal chromosome 15 from his mother, and he had seizures, severe developmental delay, and apparent prognathism. This individual might have actually had Angelman syndrome. There are several reports of sibs with unequivocal clinical PWS with cytogenetically normal chromosomes. [Burke et al., 1987; Ishikawa et al., 1987; Lubinsky et al., 1987; Orstavik et al., 1992]. Subsequent studies have demonstrated submicroscopic deletions in some of these kindreds [Saitoh et al., 1997]. The submicroscopic deletion was also present in the sibs' father in two cases [Buiting et al., 1995; Teshima et al., 1996]. In another instance, the father was not available for testing, but the deletion was present in the paternal grandmother [Reis et al., 1994]. A deletion of the PWS critical region has not been noted on routine karyotype in any cases of familial recurrence, unless a rearrangement was present.

These submicroscopic deletions are believed to disrupt an imprinting center (IC) [Buiting et al., 1995; Saitoh et al., 1997]. Mutations or deletions affecting the IC will result in an inability to reset the maternal and paternal imprints during gametogenesis. In the cases described involving the IC, submicroscopic deletions of variable length around SNRPN have been detected [Buiting et al., 1995; Reis et al., 1994; Schuffenhauer et al., 1996; Schulze et al., 1997; Sutcliffe et al., 1994] including, but not limited to the promoter and exon 1 [Saitoh et al., 1996; Ohta et al., 1999]. Individuals with PWS due to imprinting mutations are not easily distinguishable clinically from those with PWS due to a deletion or UPD [Saitoh et al., 1997]. Hypopigmentation is not present, because this is thought to be due to a deletion of the P gene locus.

The inheritance pattern seen in this family is unique to imprinting. The phenotype caused by the deletion only becomes apparent when it has passed through the paternal line. As long as the abnormality is passed through only the matrilineal line, no phenotypic effect will occur. A woman with the deletion would not be at risk for having affected children; however, her sons are at risk of having affected children. Her daughters

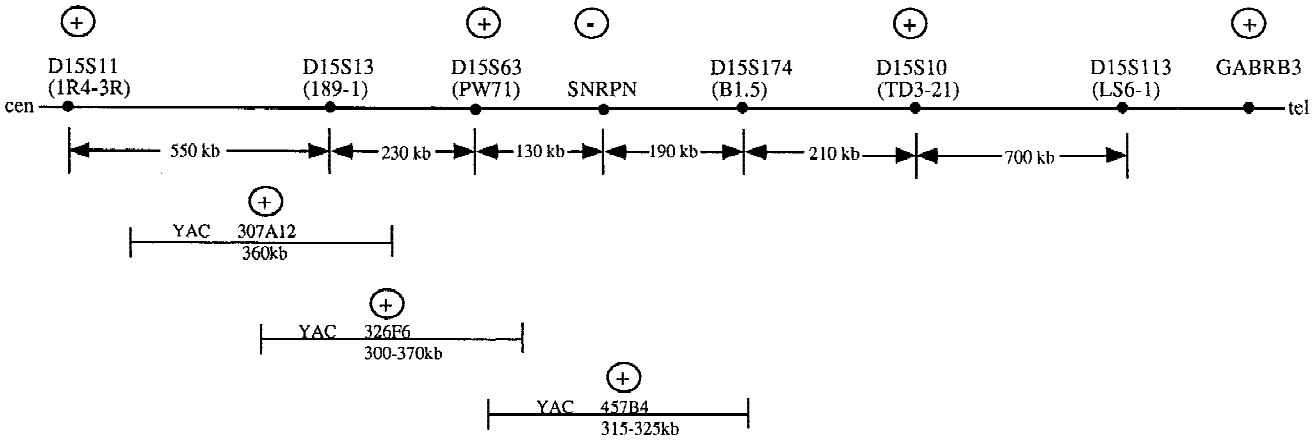

Fig. 4. Physical Map of 15q11 in the vicinity of the PWS critical region. The relevant YACS and their sizes are indicated. +, Not deleted by FISH; –, deleted by FISH.

would not be at risk, but the daughters' sons would have a risk of having children with PWS. Thus, a woman would not have affected children, but her grandchildren through her sons are at risk of showing the phenotype. This "grandmatrilineal" inheritance pattern presents new issues for genetic counseling because only the grandchildren of a carrier woman have the possibility of showing the PWS phenotype. In addition, phenotypically normal males with the deletion have a 50% recurrence risk for their children. In families where PWS is due to a deletion, the father should also be evaluated by molecular methods for the deletion. This is especially important if the deletion is not apparent using high-resolution chromosome analysis and does not involve the Angelman syndrome critical region. The size of the deletion can be estimated by using two probes in the commonly deleted region. If only SNRPN is deleted, a familial imprinting mutation must be suspected, and the potential implications for recurrence risk should be discussed. A very small imprinting center deletion may not be detectable by a cosmid probe, and in these cases, if FISH studies are normal, a more detailed molecular investigation may be required for accurate recurrence risk prediction.

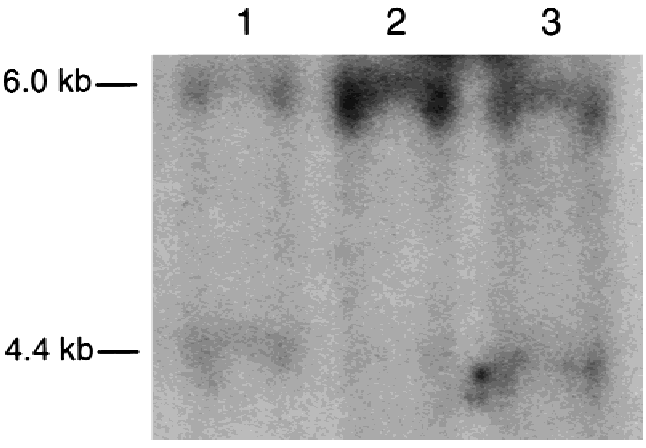

Fig. 5. DNA Methylation Studies. Genomic DNA was digested with HindIII and HpaII and hybridized with the PW71B probe. The probe detects a 6.0 kb maternal fragment and a 4.4 kb paternal fragment. (**Lane 1**) Normal control with biparental band pattern; (**lane 2**) Patient 2, affected with PWS, showing only maternal band; (**lane 3**) Clinically normal father of Patient 2, showing biparental band pattern.

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