Prader-Willi Syndrome: Genetic Tests and Clinical Findings

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ABSTRACT

Here we describe the genetic studies performed in 53 patients with the suspected diagnosis of Prader-Willi syndrome (PWS). PWS is characterized by neonatal hypotonia, hypogonadism, delayed psychomotor development, hyperphagia, obesity, short stature, small hands and feet, learning disabilities, and obsessive-compulsive behavior. Through the methylation analysis of the \textit{SNRPN} gene, microsatellite studies of loci mapped within and outside the PWS/AS region, and fluorescence \textit{in situ} hybridization (FISH) study, we confirmed the diagnosis in 35 patients: 27 with a paternal deletion, and 8 with maternal uniparental disomy (UPD). The clinical comparisons between deleted and UPD patients indicated that there were no major phenotype differences, except for a lower birth length observed in the UPD children. Our sample was composed of more girls than boys; UPD patients were diagnosed earlier than the deleted cohort (210/12 vs. 79/12 years); and, in the deleted group, the boys were diagnosed earlier than the girls (52/12 vs. 78/12 years, respectively).

INTRODUCTION

Prader-Willi Syndrome (PWS) is a disease that occurs with an incidence of \textasciitilde1:15–20,000 births, and is associated with developmental and behavioral problems. PWS children show neonatal hypotonia, hypogonadism, failure to thrive, hyperphagia, obesity, short stature, small hands and feet, mental retardation with learning disability, and obsessive-compulsive behavior (Prader et al., 1956; Cassidy, 1997). Different mechanisms can lead to PWS, which is due to a common genetic deficit (Glenn et al., 1997) that abolishes the expression of imprinted paternal genes. The major genetic mechanism giving rise to PWS is a paternal deletion of about the same size in the 15q11–q13 region, that occurs in \textasciitilde70% of the cases. Maternal uniparental disomy (UPD) is the second mechanism, occurring in \textasciitilde25% of affected individuals. Because the presence of two complete maternal chromosomes 15 cannot substitute for the absence of paternal alleles, it is suggested that the 15q11–q13 region is imprinted (Nicholls et al., 1989; Glenn et al., 1997). About 1–5% of patients with PWS have biparental inheritance of chromosome 15, but show abnormal methylation patterns and gene expression. These patients have a mutation in the imprinting process (Buiting et al., 1995; Dittrich et al., 1996; Saitoh et al., 1996, 1997; Ohta et al., 1999).

The test of the methylation pattern of \textit{SNRPN} exon 1 detects 95% of the PWS and 80% of the Angelman Syndrome (AS) patients, and the microsatellite analysis of loci within and outside the PWS/AS region distinguishes between the genetic mechanisms in each case (deletion, UPD, or an imprinting mutation). G-band analysis is recommended to distinguish numerical and/or structural chromosome aberrations, such as translocations, inversions, or marker chromosomes; fluorescence \textit{in situ} hybridization (FISH) techniques are useful in detecting the deletion cases, when parental DNA samples are not available, and also to identify the specific chromosome aberrations detected.

Some genes (\textit{SNURF-SNRPN}, \textit{IPW}, \textit{ZNF127}, \textit{NECDIN}) and transcripts (PAR1 and PAR5) are paternally expressed and map to the PWS/AS critical region. Therefore, they are good candidates to be involved in the etiology of PWS (Glenn et al., 1997; MacDonald and Wevrick, 1997; Saitoh et al., 1996). However, only two of them (\textit{SNRPN} and \textit{NECDIN}) have a known protein that is completely absent in PWS patients. The \textit{SNRPN} protein (SmN) is involved in RNA splicing, whereas \textit{NECDIN} is

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a nuclear protein that acts, mainly, in cerebral development (Glenn et al., 1996; Jay et al., 1997). These genes show paternal expression in humans and, map to the PWS/AS critical region in humans and to the syntenic conserved region in the central part of chromosome 7 in mice (Glenn et al., 1996; Jay et al., 1997; MacDonald and Wervick, 1997; Watrin et al., 1997; Gray et al., 1999).

PWS is presumed to be a contiguous gene disease because no patients with mutations in a single gene have so far been detected. Phenotype characterization is, therefore, important to understand the possible roles of genes in the critical region, with manifestations of the syndrome. Until now, two main clinical differences have been observed between the different classes of PWS patients: hypopigmentation, most common in deleted patients, is due to the loss of the P gene (Spritz et al., 1997), whereas increased maternal age has been associated with UPD cases (Butler, 1989; Gillessen-Kaesbach et al., 1995).

In this report we studied PWS patients diagnosed through methylation analysis of the SNRPN exon 1, microsatellites, and FISH. Clinical features of 27 deleted PWS patients are described and compared with those of 8 patients with maternal UPD.

### MATERIALS AND METHODS

#### Patients

Fifty-three patients suspected clinically of having PWS were referred to our laboratory for genetic tests. The majority of these patients were seen by at least one of us, but in some cases only a blood sample was sent to us, with a short list of clinical characteristics. The main features that prompted physicians to ask for these studies were hypotonia in infants, and obesity and mental retardation in children and adolescents.

#### Methods

**Cytogenetic studies:** Chromosome studies of patients and their parents were performed on peripheral blood lymphocytes to investigate structural and numerical alterations. The chromosomes were examined by the GTG banding technique.

**FISH** was performed using the SNRPN probe, specific for the 15q11–q13 region, and control chromosome 15 marker cosmids, which detect specific sequences in 15q22. The hybridization and immunohistochemical detection were carried out according to the manufacturer’s instructions (Oncor, Inc.). Twenty cells were examined for each FISH slide.

**DNA analysis:** Methylation analysis. DNA was extracted from peripheral blood leukocytes by standard procedures. The methylation status of the PWS/AS region was assessed by Southern blotting (Southern, 1975). Genomic DNA was digested with XbaI and NotI, separated by size on a 1.0% agarose gel, transferred to a nylon membrane, and hybridized using the probe that corresponds to a 0.6-kb EcoRI–NotI fragment that contains exon 1 of SNRPN (Glenn et al., 1996).

**Dinucleotide repeat (CA)n polymorphisms within the PWS/AS critical region.** Microsatellite analysis was performed with three markers within the critical region, 15q11–q13, 4-3RCA (D15S11); LS6-1CA (D15S113); and GABRB3CA (GABRB3). Two loci outside the PWS/AS region, D15S131 and D15S984, were also studied to distinguish between deletion and UPD. Deletion is suggested if there is biparental inheritance of these loci; isodisomy, if one maternal allele is present; and heterodisomy, if two different maternal alleles are evident. For UPD patients, we also analyzed the loci D15S41 and D15S42, localized close to the centromere, making it possible to identify the meiotic origin of the non-disjunction (Robinson et al., 1998). Multiplex PCR and polyacrylamide gel electrophoresis of 32P-end-labeled amplification products followed the protocols described by Mutirangura et al. (1993).

#### Statistical analysis

Statistical analysis was performed using the Fisher test, Student’s t-test, and Mann-Whitney’s test. Confidence levels of $\alpha = 0.05$ were used to indicate a statistically significant difference between the different classes of PWS patients (with deletion or UPD).

### RESULTS

Of 53 patients suspected of having PWS who were referred for genetic tests, the syndrome was confirmed in 35 (20 girls and 15 boys, with ages ranging from 3 months to 18 years) (Fig. 1), through methylation pattern analysis.

Microsatellite analysis of loci within and outside the PWS/AS region was performed in 30 of the 35 PWS patients, because parental samples were not available in 5 patients. We identified 18 patients with a paternal deletion, 8 with maternal UPD, and 4 patients with the absence of paternal alleles, but who were noninformative with regard to the specific genetic mechanism. In those 9 patients in which microsatellite studies were not available or informative, FISH showed a deletion at the PWS critical region. Seven cases with heterodisomies and one with complete isodisomy were identified among the 8 UPD patients. In summary, we obtained 27 deletion cases (77.2%) and 8 maternal UPD patients (22.8%).

The main clinical features of the UPD and deleted patients are summarized in Table 1.

### DISCUSSION

In this study, we diagnosed 35 PWS cases in 53 patients referred for genetic evaluation. Clinical characteristics of 27 individuals with a paternal deletion (14 girls and 13 boys), and 8 patients with maternal UPD (6 girls and 2 boys), were compared (Table 1).

Previous clinical comparisons between deleted and UPD PWS patients pointed only to differences in pigmentation, with hypopigmentation more frequent in deleted patients, and in maternal age, which has been reported to be increased in the UPD patients (Butler, 1989; Robinson et al., 1991; Gillessen-Kaesbach et al., 1995). Nevertheless, in our data (Table 1) a significant difference in the age at diagnosis between the two classes of PWS patients was observed: our children with UPD were diagnosed earlier than the deleted ones ($2^{10/12}$ vs. $7^{5/12}$ years, respectively), different from the studies of Mitchell et al. (1996)
FIG. 1. Prader-Willi patients. (a) patient at 24\,1/2 years; (b) patient at 15 years; (c) patient at 9 months; (d) patient at 11\,8/12 years; (e) patient at 18 years; (f) patient at 29\,1/2 years; (g) patient at 11\,8/12 years; (h) patient at 5 years; (i) patient at 5 years; (j) patient at 10 months. Patients a, c, h, and i are UPD cases; patients b, d, e, f, g, and j are deletion cases.
and Gunay-Aygun et al. (1997a). Within the deleted group, the boys were diagnosed earlier than the girls (5\textsuperscript{2/12} vs. 7\textsuperscript{8/12} years), respectively.

As expected, mean maternal age in the UPD group was higher than in the deleted group, because increased maternal age is associated with the meiotic non-disjunctive events that originate UPD zygotes (Robinson et al., 1993, 1998). Mean paternal age was also elevated in the UPD group; not unexpected because paternal age is usually increased in individuals with PWS.

Mean maternal age in the UPD group was 36\textsuperscript{10/12} years (range 19 to 47 years) compared to 27\textsuperscript{12/12} years (range 20 to 38 years) in the deleted group, with a statistically significant difference of 0.0022 (1). Similarly, mean paternal age in the UPD group was 38\textsuperscript{6/12} years (range 21 to 50 years) compared to 29\textsuperscript{9/12} years (range 23 to 43 years) in the deleted group, with a statistically significant difference of 0.0088 (1).

Reduced fetal activity was recorded in 71.43% (5/7) of the UPD group compared to 77.77% (14/18) in the deleted group, with a statistically significant difference of 1.0000 (2). Birth weight was also lower in the UPD group with a mean of 2521 (p3) g compared to 2693 (3<p<10) g in the deleted group, with a statistically significant difference of 0.3616 (3). Birth length was also lower in the UPD group with a mean of 44.42 (<<p3) cm compared to 47.87 (p10) cm in the deleted group, with a statistically significant difference of 0.0298 (3).

Table 1. Clinical Findings in PWS Patients with Deletion and UPD

<table>
<thead>
<tr>
<th></th>
<th>UPD (8 patients)</th>
<th>Deletion (27 patients)</th>
<th>P\textsuperscript{a}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age of diagnosis (y) (range)</td>
<td>2\textsuperscript{10/12} (3/12 to 6)</td>
<td>7\textsuperscript{9/12} (9/12 to 18)</td>
<td>0.0152 (1)</td>
</tr>
<tr>
<td>Sex ratio (Female:Male)</td>
<td>6:2</td>
<td>14:13</td>
<td>0.0022 (1)</td>
</tr>
<tr>
<td>Mean maternal age (y) (range)</td>
<td>36\textsuperscript{10/12} (19 to 47)</td>
<td>27\textsuperscript{12/12} (20 to 38)</td>
<td>0.0022 (1)</td>
</tr>
<tr>
<td>Mean paternal age (y) (range)</td>
<td>38\textsuperscript{6/12} (21 to 50)</td>
<td>29\textsuperscript{9/12} (23 to 43)</td>
<td>0.0088 (1)</td>
</tr>
<tr>
<td>Reduced fetal activity</td>
<td>71.43% (5/7)</td>
<td>77.77% (14/18)</td>
<td>1.0000 (2)</td>
</tr>
<tr>
<td>Birth weight (g) (%)</td>
<td>2521 (p3)</td>
<td>2693 (3&lt;p&lt;10)</td>
<td>0.3616 (3)</td>
</tr>
<tr>
<td>Birth length (cm) (%)</td>
<td>44.42 (&lt;&lt;p3)</td>
<td>47.87 (p10)</td>
<td>0.0298 (3)</td>
</tr>
<tr>
<td>Hypotonia</td>
<td>100% (8/8)</td>
<td>96% (24/25)</td>
<td>1.0000 (2)</td>
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<tr>
<td>Feeding problems</td>
<td>100% (8/8)</td>
<td>96% (24/25)</td>
<td>1.0000 (2)</td>
</tr>
<tr>
<td>Developmental delay</td>
<td>87.5% (7/8)</td>
<td>100% (26/26)</td>
<td>0.2353 (2)</td>
</tr>
<tr>
<td>Weight &gt;p90</td>
<td>50% (4/8)</td>
<td>73.08% (19/26)</td>
<td>0.3884 (2)</td>
</tr>
<tr>
<td>Height &lt;p50</td>
<td>42.86% (3/7)</td>
<td>50% (12/24)</td>
<td>1.0000 (2)</td>
</tr>
<tr>
<td>OFC &lt;p50</td>
<td>37.5% (3/8)</td>
<td>68.18% (15/22)</td>
<td>0.2098 (2)</td>
</tr>
<tr>
<td>OFC 50&lt;p&lt;97</td>
<td>62.5% (5/8)</td>
<td>31.82% (7/22)</td>
<td>0.2098 (2)</td>
</tr>
<tr>
<td>Hypertelorism</td>
<td>62.5% (5/8)</td>
<td>40% (8/20)</td>
<td>0.4097 (2)</td>
</tr>
<tr>
<td>Almond shaped eyes</td>
<td>62.5% (5/8)</td>
<td>52.38% (11/21)</td>
<td>0.6968 (2)</td>
</tr>
<tr>
<td>Strabismus</td>
<td>71.42% (5/7)</td>
<td>45.45% (10/22)</td>
<td>0.3898 (2)</td>
</tr>
<tr>
<td>Narrow bifrontal diameter</td>
<td>50% (4/8)</td>
<td>86.36% (19/22)</td>
<td>0.0596 (2)</td>
</tr>
<tr>
<td>Small hands and feet</td>
<td>71.42% (5/7)</td>
<td>81.82% (18/22)</td>
<td>0.6119 (2)</td>
</tr>
<tr>
<td>Learning disabilities</td>
<td>100% (1/1)</td>
<td>100% (16/16)</td>
<td>1.0000 (2)</td>
</tr>
<tr>
<td>Seizures</td>
<td>16.67% (1/6)</td>
<td>54.54% (12/22)</td>
<td>0.1727 (2)</td>
</tr>
<tr>
<td>Behavioral problems</td>
<td>80% (4/5)</td>
<td>78.94% (15/19)</td>
<td>1.0000 (2)</td>
</tr>
<tr>
<td>Hyperphagia</td>
<td>50% (4/8)</td>
<td>72% (18/25)</td>
<td>0.3970 (2)</td>
</tr>
<tr>
<td>High pain threshold</td>
<td>40% (2/5)</td>
<td>54.54% (6/11)</td>
<td>1.0000 (2)</td>
</tr>
<tr>
<td>Skin picking</td>
<td>100% (2/2)</td>
<td>86.66% (13/15)</td>
<td>1.0000 (2)</td>
</tr>
<tr>
<td>Decreased vomiting</td>
<td>100% (2/2)</td>
<td>100% (10/10)</td>
<td>1.0000 (2)</td>
</tr>
</tbody>
</table>

\( ^{a}\)Statistical analysis with a level of confidence of \( \alpha = 0.05 \) to assume a statistically significance difference. (1) Mann-Whitney test; (2) Fisher test; (3) Student’s t-test.

Obesity and mental retardation in children, a history of neonatal hypotonia and swallowing difficulties should be present to suggest PWS diagnosis. In babies, hypotonia, hypogonadism, feeding problems, and decreased vomiting suggest the diagnosis of the syndrome. The methylation analysis, a noninvasive exam, allows one to make the PWS diagnosis, and invasive exams, like muscle biopsy and electroneuromiography, which are usually performed in hypotonic babies, are not necessary. Consequently, with early PWS diagnosis, dietary...
management to avoid obesity and physiotherapy to improve muscle tone and avoid scoliosis, can be introduced earlier. PWS diagnosis in babies and children can avoid obesity-related health problems, which can cause early death. Parents and siblings of deletion and UPD PWS patients have low genetic risks (around 1%), unless some type of chromosome aberration is disclosed in the patient. Clinical and behavioral PWS studies in populations of different genetic backgrounds are important to delineate the phenotypic and behavioral variability present in this syndrome.

ACKNOWLEDGMENTS

We thank Dr. Robert D. Nicholls for kindly provided the SNRPN probe for methylation assay. This work is supported by FAPESP and PRONEX.

REFERENCES


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Received for publication December 2, 1999; accepted July 7, 2000.