Hypotonic infants and the Prader-Willi Syndrome

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Abstract

Objective: to describe 6 patients with less than 3 years of age that were diagnosed with Prader-Willi syndrome (PWS) due to hypotonia, poor sucking, slight facial anomalies and minor abnormalities of hands and feet. PWS is a neurobehavioural disorder characterized by two distinct phases; in the first, the neonate presents variable degree of hypotonia, feeding problems with none or poor sucking; hypogonadism, characteristic facial features with almond shaped eyes, narrow bifrontal diameter and down-turned corners of the mouth. Neuropsychomotor development is delayed. Hypotonia is non progressive and tends to improve between 8 and 11 months of age. The second phase then starts and is characterized by increasing hyperphagia and obesity, among other features. Unfortunately, most PWS patients are diagnosed only after obesity is installed.

Methods: Methylation, microsatellites analysis and karyotypic studies by traditional and in situ hybridization techniques were done.

Results: A deletion of chromosome segment 15q11q13 was disclosed in 4 and maternal disomy in two patients.

Conclusion: the diagnosis of PWS is generally established after the onset of obesity. So, we suggest that the genetic analysis must be carried out in children with severe hypotonia of unknown cause, poor sucking and some facial features of PWS (small hands and feet, hypogonadism, hypopigmentation, almond eyes and narrow bifrontal diameter). This can allow the early diagnosis and avoid invasive exams necessary for neuromuscular disorder diagnosis like muscle biopsy and electroneuromiography, which frequently are associated with inconclusive results.


Introduction

Prader-Willi syndrome (PWS), a neurobehavioural disorder which was first described in 1956, is currently one of the most frequent syndromes with chromosomal microdeletions, and the most common type of obesity of genetic origin. In addition, PWS and the Angelman syndrome (AS) (delay in neuropsychomotor development, mental retardation, absence of speech, convulsions, and happy disposition) were the first human diseases acknowledged as being determined by the genomic imprinting mechanism. The incidence of PWS is approximately 1/10,000 to 1/30,000 births and it is usually sporadic, with few familial cases reported.

Although many PWS clinical manifestations result from hypothalamic deficiencies, no structural defect of the hypothalamus has been found in postmortem exams.
Therefore, the deficiency seems to be functional, but its nature remains unknown.\(^2\) During the past few years, the genetic base of PWS has been extensively investigated; still, the clinical diagnosis of this disorder is complex, considering that some characteristics change according to the age and can be present in other syndromes as well.

PWS can be defined as presenting two distinct phases.\(^4,7,8\) The first is characterized by different levels of hypotonia during the neonatal period and the early childhood years (94%). Hypotonia is nonprogressive, and it starts to improve on average between the age of 8 and 11 months. Hypotonia is also characterized by hypothermia or hyperthermia without any apparent cause, hypogenitalism (95%), poor sucking (93%), small hands and feet and minor facial anomalies. Another aspect observed is that these children rarely vomit.

When hypotonia improves, and the child becomes more alert, there is an increase in appetite and weight gain. The onset of obesity may take place between the ages of 1 and 6, with an average at 2 years\(^8,9\) and it may be a marker to signal the beginning of the second phase. This second phase is characterized by a delay in neuropsychomotor development (98%) - the child presents a delay in learning how to sit, walk, and in speech acquisition. Other characteristics that are present in this phase are hyperphagia, obesity (94%), low stature (76%), small hands and feet (83%), decreased physical activity, decreased sensitivity to pain,\(^6,10\) hypopigmentation of hair, skin and retina, learning impairment, and facial characteristics such as a narrow forehead (75%), almond-shaped eyes (75%), and strabismus (52%). Some children between 3 and 5 years of age may develop personality problems, such as depression, irritation, violence episodes, sudden mood changes, little interaction with people, immaturity, and inappropriate social behavior.\(^11\)

PWS results from the absence of paternal genes that are usually active in chromosomal segment 15q11-q13; the maternal inherited alleles are usually inactive, due to the genomic imprinting mechanism. These paternal genes may be absent as a result of different mechanisms: 75% of the PWS cases present paternal deletion of segment 15q11-q13; 20-25% present maternal uniparental disomy (inheritance of two maternal chromosomes 15);\(^12,13\) approximately 5% of the PWS cases present translocation or other structural chromosomal anomaly involving chromosome 15; around 1% of the patients (including all the families with recurrence of PWS that have been studied so far) do not present deletion or uniparental disomy, but instead present a microdeletion in the imprinting controller center, called imprinting center, located in segment 15q11-q13.\(^6,14,17\)

In this work, we describe the clinical characteristics and the genetic diagnosis of six patients younger than 3 years of age, emphasizing the importance of an early PWS diagnosis.

Patients and Methods

Patients

The patients were referred to the Genetic Counseling Service (IB-USP) with hypotonia, poor sucking and slight anomalies on the face and extremities (Figure 1). Consent for publication of the photographs was obtained from all parents.

Genetic study

The diagnosis of PWS was established through analysis of the exon 1 methylation pattern in the SNRPN gene, located in the PWS and AS critical region, using the Southern blot technique.\(^18\) The genetic mechanism was determined through the analysis of the chromosome 15 microsatellite segregation standard, using the polymerase chain reaction (PCR) technique.\(^19\) G-banding karyotype and fluorescent in situ hybridization (FISH).

Results

The patients’ clinical characteristics are summarized in Table 1. The karyotypic analysis was carried out in five out of six patients, with results in all of them. Regarding molecular results, all six patients presented a typical PWS methylation pattern, confirming the clinical suspicion. The analysis of microsatellites, along with FISH results, revealed that, out of six patients with PWS, four presented deletion of segment 15q11q13, and two presented maternal uniparental disomy.

Discussion

The differential diagnosis of hypotonia in infants includes neuromuscular diseases such as infant spinal amyotrophy and congenital myopathies. For the diagnosis of these diseases, the performance of electromyography and muscle biopsy is indicated. The performance and interpretation of these invasive exams is sometimes difficult, and they may lead to wrong diagnoses. In PWS, muscle biopsy can reveal atrophy of type-II fibers, but this finding is not specific.

The karyotype rarely defines a diagnosis of PWS or AS. Currently, the most efficient method to diagnose these diseases is a molecular test that determines the progenitor-specific methylation pattern in the PWS/AS region, using Southern blot and hybridization with probes that are sensitive to methylation of the SNRPN and PW71 loci.\(^6,20-23\) In patients with suspicion of PWS, a normal methylation pattern eliminates the possibility of PWS with 95% certainty.

Although the prevalence of PWS in children with hypotonia is not known, the methylation test has to be considered for the differential diagnosis, mainly among infants presenting serious hypotonia of unknown cause.
Gillessen-Kaesbach et al. tested 65 children between 0 and 12 months of age with idiopathic hypotonia and detected 29 PWS cases (45%). Those authors emphasize that although this frequency is probably overestimated, due to an investigation bias, the methylation test should be performed in this group of patients, considering that it is non-invasive and extremely effective to diagnose PWS.

The prenatal diagnosis through the study of the methylation pattern should be considered in families with rearrangements in chromosome 15, in women who already had a child with the syndrome, and in patients with trisomy of chromosome 15 on chorionic villus culture and a normal chromosome count on amniocentesis, since it is known that advanced maternal age is associated with cases of uniparental disomy 15, due to its association with meiotic errors. In our sample, we also observed that the mothers were older in uniparental disomy cases.

The search for the genetic mechanism that causes PWS is important for the genetic counseling of parents and relatives. The risk for deletion and disomy is low, around 1%; the only high risk (50%) is associated with rare occurrences of mutations and translocations in the imprinting mechanism.

The early diagnosis of PWS is important because it gives the parents the opportunity of providing adequate diets and stimulating appropriate eating and exercise habits, so as to reduce the problems related to obesity, e.g. diabetics, hypertension, and respiratory problems, which are the main causes of death among these individuals during adolescence. In addition, children and teenagers with PWS present a developmental delay in several fields, and an early diagnosis prompts the parents to look for professional help (teachers, pedagogues, physical therapists, and speech therapists).

During adolescence, the family may lose control over the child’s diet, since teenagers seem to use their intelligence and shrewdness to obtain food, becoming aggressive when it is denied to them. These individuals may eat leftovers, pet food, and some of them even eat dirt; some children may develop “psychotic” behaviors. Experience shows that psychological support to the patient and to the parents and
Table 1 - Clinical characteristics and complementary exams of PWS carriers

<table>
<thead>
<tr>
<th>Patient</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
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<td>Sex</td>
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<td>M</td>
<td>M</td>
<td>F</td>
<td>F</td>
<td>M</td>
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<tr>
<td>Age (months)</td>
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<td>10</td>
<td>20</td>
<td>9</td>
<td>10</td>
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<tr>
<td>Mother’s age (years)</td>
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<td>28</td>
<td>30</td>
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<td>48</td>
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<tr>
<td>Father’s age (years)</td>
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<td>Normal</td>
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<td>Decreased</td>
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<tr>
<td>Birth weight (g)</td>
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<td>2680</td>
<td>3025</td>
<td>2440</td>
<td>1780</td>
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<td>Length (cm)</td>
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<td>47</td>
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<td>51</td>
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<td>Hypotonia</td>
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<td>+</td>
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<td>+</td>
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<tr>
<td>Poor sucking</td>
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<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
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<td>Delay in neuropsychomotor development</td>
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<td>+</td>
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<tr>
<td>Weight</td>
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<td>10%</td>
<td>25×P&lt;50%</td>
<td>50%</td>
<td>90×P&lt;97%</td>
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<tr>
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<td>75%</td>
<td>75%</td>
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<td>Cephalic perimeter</td>
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<td>25×P&lt;50%</td>
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<td>&lt;2.5%</td>
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<td>Almond-shaped eyes</td>
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<td>+</td>
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<td>+</td>
<td>-</td>
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<td>+</td>
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<td>Narrow forehead</td>
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<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
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<tr>
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<td>+</td>
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<td>NP</td>
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<td>type II FA</td>
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<td>NP</td>
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<td>Genetic mechanism</td>
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<td>Deletion</td>
<td>Deletion</td>
<td>Deletion</td>
<td>UPD</td>
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</tbody>
</table>

+ = presence of characteristic  - = absence of characteristic  0 = data not available  NP = not performed  type II FA = type II fiber atrophy  UPD = uniparental disomy  * = prenatal exam performed, showing chromosome 15 trisomy on chorionic villus sampling and normal complement on amniocentesis

brothers/sisters should begin during childhood and continue throughout adulthood, when the major problem is weight and behavior control, with the occurrence of irritation periods, and sometimes of psychotic episodes.

Since in our setting the diagnosis of PWS is generally established after the beginning of obesity, we suggest genetic testing for this disease be requested in newborns and infants presenting hypotonia, poor sucking, and some of the phenotypic characteristics of PWS (small hands and feet, signs of hypogonadism, hypopigmentation in relation to relatives, almond-shaped eyes, narrow forehead). This may help the establishment of an early diagnosis, decreasing the need for more invasive diagnostic resources, sometimes of difficult interpretation, such as electroneuromyography and muscle biopsy.

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References


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