CLINICAL AND ENDOCRINE ASPECTS OF FIVE PRADER WILLI PATIENTS

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Abstract
Prader Willi syndrome is a complex disease caused by the lack of expression of paternally inherited imprinted genes on chromosome 15q11.2-q13. Typical clinical features are hypotonia and feeding difficulties in infancy, followed by hyperphagia and progressive obesity, distinctive dysmorphic features, intellectual disability and behavioural problems. In this paper we present clinical, metabolic and endocrine aspects in five genetically confirmed patients with PWS. Data about thyroid dysfunction, GH deficiency, adrenal insufficiency, and LH/FSH disorder caused by hypothalamic dysfunction in PWS were collected and analyzed. Cardiovascular metabolic profile was also assessed, based on plasma lipids, blood glucose, HbA1c values, and measurements of body weight and blood pressure. Clinical features present in all our patients were marked hypotonia and feeding difficulties in infancy, obesity, dysmorphic face, viscous saliva, small hands and feet, intellectual disability and characteristic behaviour. Adrenal function appeared to be normal in all patients; mild hypothyroidism was identified in one patient; sex development abnormalities were present in three patients and GH levels were within lower normal range in all patients. GH therapy was initiated in two patients, both with unevolutive skeletal anomalies, with good results and no side-effects. Only one patient had a normal lipid profile, underlying the importance of early detection and treatment of cardiovascular risk factors. Our study also illustrates the challenges raised by some features very rarely described in PWS (Blount disease and multiple allergies).

Key words: Prader Willi syndrome, hypothalamic - pituitary insufficiency, GH therapy, Blount disease, multiple allergies.

INTRODUCTION

Prader Willi syndrome (PWS) is a complex disease caused by the lack of expression of paternally inherited imprinted genes on chromosome 15q11.2-q13. The main molecular mechanisms are paternal deletion in about 70% of subjects, maternal uniparental disomy (UPD) in about

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25% and translocations or imprinting centre mutations in 1-3% (1). PWS has a characteristic phenotype with newborn hypotonia and feeding difficulties during infancy, hyperphagia with progressive obesity during childhood, distinctive dysmorphic features, intellectual disability (ID) and behavioural problems. Consensus criteria for clinical diagnosis of PWS were first established in 1993 by Holm (2) and are used as a screening test for identifying appropriate patients for genetic tests. Patients with UPD have an increased risk of psychosis (3) and autism spectrum disorders (4), but the facial dysmorphism is mild (5, 6), the verbal IQ is higher (7) and jigsaw puzzles skills are less developed compared with patients with deletion (8).

Most cases of PWS occur sporadically (9). The recurrence risk depends on the molecular mechanism that leads to PWS: usually less than 1%, except for inherited mutation in imprinting centre (up to 50% risk) and parental translocation (up to 25% risk) (1). Associations between PWS and sex chromosome aneuploidies are very rare and the chance for their occurrence is very low. Four studies reported an association between PWS and 47, XXX syndrome (10-13).

PWS patients have hypothalamic - pituitary insufficiency which can lead to the development of multiple endocrinological disorders such as central adrenal insufficiency, GH deficiency, LH/FSH abnormalities and central hypothyroidism.

The main objectives of our study were to evaluate clinical, metabolic and endocrine aspects in five genetically confirmed patients with PWS.

**PATIENTS AND METHODS**

The study included five patients (four females and one male, aged 5.5 - 19 years), admitted into our department with the suspicion of genetic obesity. Written informed consent from parents was obtained before detailed evaluation. The patients were selected according to Holm’s criteria and genetically confirmed in our laboratory by Methylation-Specific Multiplex Ligation-dependent Probe Amplification (MS-MLPA using SALSA ME028-B1 Prader Willi/Angelman probe mix). Patients for which MS-MLPA showed a deletion were subsequently tested by FISH (Vysis Prader-Willi/Angelman Region Probe - LSI D15S10 Spectrum Orange/CEP 15 - D15Z1 Spectrum Aqua/PML Spectrum Green Probe).

Anthropometric measurements, detailed physical examination and blood pressure measurement were performed for all patients. Medical and surgical history was recorded. Fasting blood samples were collected for determination of glucose, HbA1c, total cholesterol, HDL-cholesterol, triglycerides and hormones such as basal GH, IGF1, TSH, fT4, cortisol, FSH, LH and sex hormones using commercially available chemiluminescent immunoassays (IMMULITE 1000). Provocative GH stimulation and oral glucose tolerance tests were not performed because of the food seeking behaviour and the range of cognitive abilities in patients with PWS.

Bone mineral density (BMD) of lumbar spine (L1-L4) and femoral neck
was assessed using Dual Energy X-ray Absorptiometry (DXA), according to the standard procedure using a Hologic Delphi W instrument. The patients’ BMD results were compared with data matched for age and sex and reported as Z-scores. The World Health Organization definition of osteoporosis/osteopenia was applied (14). Bone X-rays were performed if skeletal abnormalities were present.

Polysomnographic studies were performed in all patients.

RESULTS

Features included in Holm’s diagnostic criteria and present in all our patients were: marked hypotonia and feeding difficulties in infancy, obesity (weight between +18.4 SD and +5.3 SD), dysmorphic face, viscous saliva, small hands and feet, ID and characteristic behaviour (hyperphagia, stubbornness, repetitive skin picking, repetitive questioning and insistence on routine, high pain threshold) (Table 1). Patient 1 associated macrocephaly (+3 SD), genu varum and premature pubarche (at 7 years of age). Patient 5 presented primary amenorrhea.

MS-MLPA revealed presence of two methylated copies and normal dosage at 15q11.2-q13 in four patients (patients 1-4) (Fig. 1) and a type II heterozygous deletion (between breakpoint 2 and breakpoint 3) within 15q11.2-q13 in patient 5 (Fig. 2). The deletion was confirmed by FISH test.

Blood pressure was normal for all cases. Biochemical evaluations revealed normal glucose and normal HbA1c. Lipid assessment revealed: hypertriglyceridemia (patients 1 and 2), hypercholesterolemia (patient 5), combined hyperlipidemia (patient 4) and normal values (patient 3).

Endocrine evaluation:

- Hypothalamic–pituitary–GH axis: in all patients basal GH and IGF1 levels were lower, but still within normal limits for age and sex, according to the reference values of the assay. Patients 1 and 2 received GH therapy at a dose of 0.05 mg/kg/day administered daily by subcutaneous injection. GH treatment was not initiated in patients 3, 4 and 5 due to sleep apnoea;

- Hypothalamic–pituitary–thyroid axis: TSH and fT4 levels and thyroid ultrasound were normal in patients 2-5; for patient 1 TSH was 4.4 μUI/mL (0.4 – 3.45) and fT4 was 1.3 ng/dL (0.8-2);

- Hypothalamic–pituitary–gonadal axis: FSH and LH levels were in the normal range for sex and age. Sex hormones levels (testosterone, estradiol) were normal for age and sex in patients 1-4 and low, showing hypogonadotropic hypogonadism (prepubertal range) in patient 5, aged 19;

- Hypothalamic-pituitary-adrenal axis: basal cortisol levels were within normal range, but no dynamic assays were performed;

- BMD: mean Z-scores were reduced for the spine, but normal for the hip in three patients.

Skeletal X-rays and orthopaedic examination revealed severe sinistro-convex scoliosis of the dorsal spine (patient 5), mild scoliosis (patient 2) and tibia vara - Blount disease (patient 1).
revealed moderate obstructive sleep apnoea for patient 4 and mild mixed sleep apnoea for patients 3 and 5.

Psychological evaluation showed mild ID in patients 1-4 and severe ID in patient 5. Further imagistic investigations (MRI) showed that patient 5 associates frontal cortical atrophy with secondary seizures and patient 1 had hydrocephaly with EEG abnormalities in mesodiencephalic areas.

Patient 5 associated also
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**Table 1.** Consensus diagnostic criteria for PWS (2, 6)

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<tr>
<td>Age</td>
<td>10 y</td>
<td>11 y 8 mo</td>
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<td>Sex</td>
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**Major criteria (1 point each)**
- Neonatal/infantile hypotonia/poor suck
- Feeding problems and failure to thrive as infant
- Weight gain at 1-6 years/obesity/hyperphagia
- Characteristic dysmorphic facial features
- Genital hypoplasia/cryptorchidism/pubertal delay/hypogonadism
- Developmental delay/ID

**Sum of the major criteria points**
- Patient 1: 5
- Patient 2: 6
- Patient 3: 5
- Patient 4: 5
- Patient 5: 6

**Minor criteria (1/2 point each)**
- Decreased foetal movement or infantile lethargy
- Characteristic behaviour problems
- Sleep apnoea
- Short stature for family by 15 years of age
- Hypopigmentation for the family
- Small hands and feet for height
- Narrow hands with straight ulnar border
- Esotropia, myopia
- Thick, viscous saliva
- Speech articulation defects
- Skin picking

**Sum of the minor criteria points**
- Patient 1: 3
- Patient 2: 3
- Patient 3: 3.5
- Patient 4: 3
- Patient 5: 5

**Total sum**
- Patient 1: 8
- Patient 2: 9
- Patient 3: 8.5
- Patient 4: 8
- Patient 5: 11

Clinical diagnosis: 5 points (at least 4 of them from major criteria) at age < 3 years; 8 points (at least 5 of them from major criteria) at age > 3 years.

*na - not applicable due to the young age of the patients.

Compound myopic astigmatism and multiple food and drug allergies. The multiple drug allergies included recurrent antihistamine-induced urticaria (after taking cyproheptadine and clorpheniramine), allergic reactions to topiramate and ibuprofen, anaphylactic reactions to penicillin, penicillin derivatives and metamizole. Furthermore, she had pollen, mold and detergent induced rhinoconjunctivitis and also allergies to insect stings (bees).
**DISCUSSION**

**GH deficiency**

PWS patients are characterized by low growth velocity, obesity, reduced lean body mass and decreased bone density, as well as delayed bone maturation, features indicative of deficient GH production (15, 16). More than 85% of patients with PWS have low levels of basal GH and IGF-I and, depending on the stimulation test used, 40–100% of children fulfil the criteria for GH deficiency (17). GH secretory pattern is different in patients with UPD (lower basal GH values, poor answer to GHRH or arginine test) compared to patients with deletion, suggesting a better response to GH therapy in UPD patients (18). GH treatment in PWS patients improves short stature, body composition, fat utilization and motor function (19, 20). Growth-promoting effects of GH therapy seem to be more prominent in hyperleptinemic GH deficient children (21).

Although all our patients had normal growth parameters (height and height velocity) and GH levels within lower normal range, in accordance with literature data (22), we considered beginning GH therapy. For GH treatment we used as exclusion criteria severe obesity, uncontrolled diabetes mellitus, untreated sleep apnoea, active cancer and psychosis (1, 23). Scoliosis was not considered a contraindication to GH treatment because neither its incidence nor its rate of progression are influenced by this treatment (23, 24). Therefore, patients 3, 4 and 5 (who presented sleep apnoea) did not receive treatment. There is no consensus on age of starting GH treatment, although the benefit of treating before the onset of obesity, which often begins by 2 years of age, is documented (23). Patients 1 and 2 received GH therapy from 5.5 years of age and 8 years 8 months, respectively. We used a dose of 0.05 mg/kg/day, calculated using actual weight, according to previous guidelines (25). New consensus guidelines for GH therapy in PWS recommend starting with a daily dose of 0.5 mg/m²/day subcutaneously with subsequent adjustments toward 1.0 mg/m²/day every 3–6 months according to clinical response (23). GH therapy in conjunction with dietary, environmental and lifestyle interventions showed significant improvement of mental/motor development and no adverse effects, despite the late start of GH therapy (22).

Recent studies have shown that long-term GH treatment in adults with PWS has favourable effects on abnormal body composition without clinically significant side effects (26, 27). If no exclusion criteria are present, when arrived at adult age our patients should receive a starting dose of 0.1–0.2 mg/day based on age, presence of edema, prior GH exposure and sensitivity, and concomitant oral estrogen use (23).

From the best of our knowledge, there is only one case of PWS who associates Blount disease reported in the literature (28). One of our cases who received GH treatment (patient 1) had Blount disease, and showed no skeletal anomalies progression during one year therapy. The GH treatment was stopped to perform
orthopedic surgery for Blount disease.

**Thyroid function**

Previous studies reported a frequency of hypothyroidism in PWS patients varying widely, from 2% (29) to 25% (30, 31). Only one of our cases had subclinical hypothyroidism (patient 1) and received low dose substitute therapy (levothyroxine 25 μg/day) due to metabolic particularities. Hypothyroidism in PWS patients may be congenital or of late onset (32), therefore the levels of TSH and fT4 need to be monitored at birth and thereafter yearly, or every six months during GH therapy (1).

**Gonadal function**

Previous studies reported isolated premature pubarche, without other signs of puberty, in approximately 14 – 20% of PWS patients, probably due to early maturation of zona reticularis of the adrenal glands (33, 34). In our study only patient 1 (who presented hydrocephalus) had premature pubarche. Children with hydrocephalus may have frequently short stature and precocious puberty (35), therefore the premature pubarche in our patient may be also due to the damage of the hypothalamus or pituitary gland caused by increased intracerebral pressure.

Delayed or incomplete pubertal development, including primary amenorrhea, are more common in PWS patients (15). Consistent with this data, patient 5 presents delayed puberty.

Cryptorchidism is present in 80-90% of PWS boys and should be treated in the first year of life to prevent germ cells destruction (30). The most efficient treatment is surgery, but some authors recommend human chorionic gonadotropin as first intention therapy in order to avoid general anaesthesia risk existing at PWS children (36). In our only male (patient 2) cryptorchidism was treated in the second year of life, by surgery, without anaesthetic incidents. At some moment in the future our patients will need sex steroid replacement in order to induce or maintain puberty. There is no agreement on the most appropriate therapy for puberty development in patients with PWS, options depending on the local team experience and pharmaceutical market possibilities. In adolescents and adults with PWS, the main objective of this therapy is prevention of osteoporosis (22).

**Adrenal insufficiency**

Our patients had normal basal cortisol and no signs of adrenal insufficiency. According to a recent study adrenal insufficiency in PWS patients has a low prevalence (7.5%), but clinicians are advised to test the patients for central adrenal insufficiency. The same study also showed that basal cortisol was closely correlated with adrenal response to stimulation, indicating that its measurement may be helpful in selecting patients for low-dose short Synacthen test (37).

**Bone mineral density (BMD)**

Three of our patients presented low Z-scores, suggestive of osteopenia/osteoporosis. After one year therapy with vitamin D and calcium supplements, Z-scores increased notably (from -3.6 to -2.2 and from -3.7 to -2.4 in two patients, and from -1.9 to -1.2 in one patient). Reduced BMD in PWS patients can be related to hypogonadism, low GH hormone level and low physical activity.
Salles et al. showed that osteopenia is not present at early stage in PWS patients, but develops later, specially during puberty (38). Therefore, we suggest that all PWS patients must be periodically investigated by using DXA.

Additional medical issues

Body composition studies in PWS patients showed increased body fat from infancy to adulthood (39, 40), which is considered a risk factor for cardiovascular disease and diabetes mellitus type 2. Our patients presented dyslipidemia, whereas blood pressure and glucose homeostasis were normal, which is consistent with data reported by previous studies (41). Considering that 25% of adults with PWS have been reported with type 2 diabetes mellitus (mean age of onset about 20 years) (42) and hypertension may be present in up to 38% in adults (43), the patients must be periodically reevaluated. The management of type 2 diabetes mellitus in PWS patients is difficult due to uncontrolled food intake. New studies suggest single dose of glucagon-like peptide 1 receptor agonists as a novel therapy, considering its potential effects on glycemic control, delayed gastric emptying and increasing satiety (44).

Food allergy and atopy is uncommon in patients with PWS, the first case being reported in 2004 (45). Patient 5 had multiple allergies which, associated with characteristic food seeking behaviour, increase the risk of anaphylaxis. Patient 5 had also recurrent antihistamine-induced urticaria, which was rarely reported. No precise mechanism had been established, but some authors consider this an immunoglobulin E–mediated reaction (46).

Epileptic seizures are found in 18–26% of PWS patients (47, 48). One of our cases (patient 5) associated epilepsy, challenging aspect due to the fact that this patient associated also multiple allergies, including to some anticonvulsivant drugs.

Genotype - phenotype correlations performed in PWS patients revealed that hypopigmentation and epilepsy are seen primarily in patients with deletion (22, 47). Four of our patients presented abnormal methylation profile and one presented a deletion, but we did not observe hypopigmentation in any of our patients. Other studies showed that patients with UPD have higher verbal intelligence scores and less maladaptive behaviors compared with patients with deletions (4, 49). Considering that the vast majority of PWS patients with abnormal methylation profiles are due to UPD, the mild ID which was diagnosed in patients 1–4 can be explained by the involvement of this molecular mechanism.

In conclusion, our study aimed to evaluate clinical, metabolic and endocrine aspects in five patients with PWS. PWS is a complex disease, associated with endocrine dysfunctions, that requires a multidisciplinary approach. Marked hypotonia and feeding difficulties in infancy (found in all our patients) are early evocative features for PWS. Early diagnosis of PWS is essential for starting GH therapy and for appropriate genetic counseling. Although we started relatively late the GH therapy and it was administered to patients with unevolutive skeletal anomalies, we
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observed significant improvement in mental development and in body habitus and no adverse effects. Our study also illustrated the challenges raised by some features very rarely described in PWS (Blount disease and multiple allergies).

Conflict of interest
We declare that there is no conflict of interest.

Acknowledgment
Elena Braha and Corin Badiu contributed equally to this work. We thank the subjects with PWS and their families for participating in the study.

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