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Does the Genetic Cause of Prader-Willi Syndrome Explain the Highly Variable Phenotype?

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ABSTRACT

Introduction: *Prader-Willi syndrome (PWS) is characterized by extensive clinical and genetic variability caused by lack of expression of imprinted genes of the chromosomal region 15q11.2-q13. The genotye-phenotype correlation has not been yet fully elucidated.*

Aim: *To analyze these correlations in order to determine the role of specific geneic alterations in the development of clinical symptoms in PWS.*

Material and method: *We retrospectively analyzed data routinely collected as part of the clinical care of 52 patients with clinical suspicion of PWS. FISH test was performed in all patients; in case of negative results, methylation test was performed.*

Results: *PWS was confirmed in 35 patients that were divided in two groups according to the genetic cause of PWS: group A-21 patients with 15q11-q13 region deletion, mean age at evaluation 8.1 years (SD= 5.6) and mean of clinical score 9.4 ± 1.8; group B-14 patients with positive methylation test, with mean age at evaluation 6.7 years (SD= 4.6) and mean of clinical score 10.1 ± 1.9. Facial dysmorphism and neonatal hypotonia were present in all evaluated patients; while, higher frequency of major and minor PWS criteria were noted in the group A. Onset of hyperphagia, was around the age of 2 years in most patients, however one patient from group B had normal eating behavior and normal weight beyond age 5 years.*

Conclusion: *In our study, the various genotypes did not seem to explain the difference in phenotype in PWS patients. We found a delayed time until diagnosis in these patients, although all had neonatal hypotonia and other suggestive phenotypic features, underlining once more the need for increased awareness of this syndrome, as well as easier accessibility to genetic counseling.*

Keywords: Prader Willi syndrome, hyperphagia, obesity, genotype, chromosome 15, uniparental disomy

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Legend:
CA- central apneas
DNA- Deoxyribonucleic acid
FISH- Fluorescence *in situ* hybridization
GH- Growth hormone
Hyp- hypopneas
MA- mixed apneas
OA- obstructive apneas
PWS- Prader Willi syndrome
SD- Standard deviation

INTRODUCTION

Prader-Willi syndrome (PWS) is a rare genetic disease with multisystem involvement, the most common form of life-threatening syndromic obesity (1). It has a prevalence of 1: 10,000 to 1: 30,000 newborns and it affects both males and females with no race particularities (1). One important feature of PWS is the high phenotypic variability (2). The most common clinical features are diminished fetal movements, hypotonia, failure to thrive and later, obesity, intellectual disability, short stature, hypogonadotropic hypogonadism, strabismus and small hands and feet. Genetically, the disease is caused by a microdeletion or disruption of genes in the proximal arm of paternal chromosome 15 or maternal uniparental disomy of chromosome 15 (3). Chromosome 15 represents more than 3% of the total DNA in cells and contains approximately 700 genes coding proteins. The PWS corresponding part of chromosome 15 is located on 15q11.2-q13 (4). PWS is the first disease attributed to a genomic imprinting. In such diseases, the genes are differentially expressed based on maternal or paternal origin. Most cases of PWS involving deletions or unbalanced translocations; maternal disomy is sporadic. Approximately 65-70% of PWS cases are due to a microdeletions of 15q11-13 band on paternal chromosome 15 (4). Uniparental disomy appears when both copies of a chromosome were received from one parent; it occurs in about 25% of PWS cases. 5% of cases are caused by isolated mutations of the imprinting center with high risk of recurrence (5). Other causes could be gene mutation (<0.1%) or balanced translocation (0.1%). There are also some cases (1%) with unknown cause, Prader-Willi like phenotypes.

PWS is a multisystem disease, with significant phenotypic features changes influenced by many factors: patient age, early diagnosis, early therapeutic attitude, the genetic causes of disease.

Depending of the length of deletion, symptoms severity is different. If the deletion is small, the characteristic features (neonatal hypotonia, appetite) could be milder (3).

Clinical score for PWS involves a number of diagnostic criteria. Holm and collaborators have established major clinical criteria (noted with 1 point each), and the minor criteria (0.5 points each) (6). A clinical score of 5 points for children younger than 3 years (at least 3 major criteria present) and a score of 8 points for children over 3 years (5 points obtained from the major criteria) is required for a clinical diagnosis of PWS (Table 1).

Major criteria (1 point each)	Minor criteria (0.5 points each)
Neonatal/infantile hypotonia and poor suck	Decreased fetal movement and infantile lethargy
Feeding problems and failure to thrive as infant	Typical behavior problems
Weight gain at 1 to 6 Obesity Hyperphagia	Sleep apnea
Characteristic dysmorphic Facial features	Short stature for family by 15 years
Small genitalia Pubertal delay Insufficiency	Hypopigmentation for the Family
Developmental delay/ Intellectual disability	Small hands and feet for height
	Narrow hands, straight ulnar border
	Esotropia Myopia
	Thick, viscous saliva
	Speech articulation defects
	Skin peeking

TABLE 1. Consensus diagnosis criteria for PWS (Holm et al 5)

The supportive criteria (no points) include high pain threshold, decreased vomiting, ineffective thermoregulation, scoliosis, kyphosis, early adrenarha and osteoporosis, unusual ability to build puzzles, no changes in neuromuscular tests.

The syndrome presents high clinical variability. Although several studies evaluated the relation between the genotype and phenotype, it has not been fully elucidated. Thus, the study aims to analyze genotype-phenotype correlations in our center's experience and review the literature, in order to determine the role of specific gene mutations in the development of clinical symptoms of the disease. ❑

MATERIAL AND METHOD

We performed a retrospective study that analyzed data routinely collected as part of the clinical care for the patient with PWS. We evaluated 52 patients, aged between 2 months and 17 years, 27 girls (52%) and 25 boys (48%) with a clinical diagnosis of PWS. We excluded the patients with negative methylation test from the study. The main phenotypic features were analyzed according to the international clinical diagnostic criteria for PWS (minor criteria, major criteria and supportive criteria) and correlated with genotype. The patients younger than 1 year were excluded from certain correlations (assessment of language disorders, sleep disorders, weight gain after the age of 1 year, spine abnormalities). The information about the pregnancy and postnatal evolution, and the onset of hyperphagia and obesity were collected from interviews with the parents. We measured their weight and height and compared the results with the standardized values for age and sex. The laboratory tests included a complete evaluation of the carbohydrates and lipids metabolism and hormonal status. The multidisciplinary team had an important role to diagnose the endocrine problems, ocular abnormalities, the intellectual disabilities and language disorders. We performed specific FISH test for all patients. FISH technique uses fluorescent probes and can detect only the microdeletion of 15q11-13 band on chromosome 15, but is no informative for other causes of PWS. For FISH analysis were used XL SNRPN probes (MetaSystems) according to manufacturer protocol. Slides were analysed on a Zeiss Axio Imager 2 microscope and at least 100 nuclei and 20 metaphases were counted. The methylation test was the second step for molecular diagnosis of PWS, it was applied to the patients without microdeletion of 15q11-q13 region (FISH negative). The DNA probes were isolated from peripheral blood collected on EDTA tubes. The methylation test analysis was used in order to establish the methylation pattern and subsequently detects the parent of origin (7). The identification of maternal disomy in chromosome 15 confirmed the diagnosis of PWS. In evolution, all the patients have a complete medical evaluation every 3 month in our center and at least one patients group meeting *per* year on Rare Diseases Center from Zalau. We also were looking for associated pathology with known or current treatments. For sleep disorders, we used Polysomnography, a detailed overnight sleep study which monitors ventilatory variables (movement of chest wall and airflow at mouth and nose level), arterial oxygen saturation (by finger/ear pulse oximetry) (8, 9). Apnea was defined as a reduction of more than 90% of nasal flow and hypopnea a reduction between 50-80% with 4% oxygen desaturation, estimating apnea-hypopnea index. Central apnea was noted when chest movements were absent, for obstructive apnea the chest movements were maintained. Language disorders were considered difficulties in words articulation.

For data analysis, we applied chi squared test and student t test, using R statistical program. ❑

ETHICAL CONSIDERATIONS

We obtained the written informed consent after discussion with each parent about the suspected diagnostic, the nature and purpose of the proposed procedures and evaluations, the risks and benefits of procedures and alternatives.

The study procedures consisted retrospective analysis of medical routine care, the parent/legal guardian of each participant provided verbal consent for inclusion of children's medical data. The study respects the Helsinki Declaration regarding confidentiality of data collected. The authors obtained appropriate patient consent forms for the use of patient photographs. ❑

RESULTS

PWS was confirmed by FISH or methylation test in 35 patients, while for 18 the suspicion of PWS was not confirmed by using the above mentioned tests. In 21 patients, 9 girls (43%) and 12 boys (57%) a 15q11-13 microdeletion was established by FISH (Figure 1) and those patients were included in group A; 14 patients, 8 girls (57%) and 6 boys (43%) had a positive methylation test (group B). The mean age for group A was 8.11 years and SD 5.6, average clinical score and SD for the same group was 9.4 ± 1.8. In group B, the mean age at diagnosis was 6.7 years and SD of 4.6, clinical score average and SD of group B was 10.1 ± 1.9. All patients from group A showed spe-

Major criteria distribution			
	Group A (%)	Group B (%)	Differences between groups (p-values) - chi squared test
Facial dysmorphism	100%	100%	0.16
Neonatal hypotonia	100%	100%	0.16
Rapid and excessive weight gain between 1 and 6 years	100%	92%	0.01
Hypogonadism	71%	43%	0.09
Development delay/ intellectual disability	94%	100%	0.04
Minor criteria distribution			
	Group A (%)	Group B (%)	Differences between groups (p-values)
Hair and skin hypopigmentation	38%	29%	0.56
Small hands and feet	81%	100%	0.02
Viscous saliva and angular cheilitis	52%	93%	0.01
Ocular abnormalities (esotropia and myopia)	52%	43%	0.58
Sleep disorders	83%	75%	0.57
Language disorders	89%	100%	0.23

TABLE 2. PWS criteria distribution in studied patients

cific facial dysmorphism for PWS (p = 0.16): almond shaped eyes, down turned corners of the mouth, thin upper lip, narrow bifrontal diameter. All the evaluated patients presented neonatal or infantile hypotonia and poor sucking reflex; the micro deletion group presented a higher rate of feeding difficulties and need for specialized assistance. Rapid and excessive weight gain between 1 and 6 years was present in all patients from group A and in 92% from the group B (p = 0.01). The hyperphagia caused excessive weight gain, with onset around the age of 2 years in most patients. They developed morbid obesity, a characteristic PWS feature. Fat distribution was similar for both patient groups.

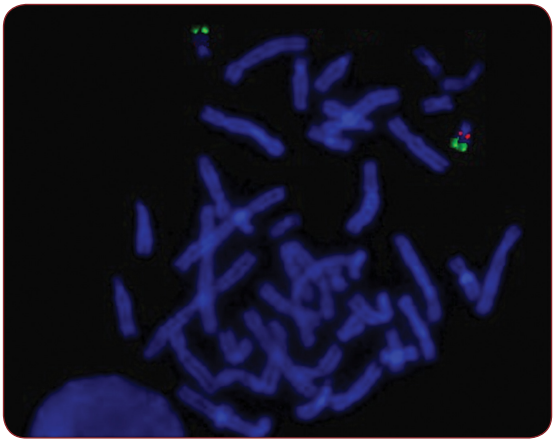

FIGURE 1. Positive FISH test: 15q11q13 microdeletion was confirmed

Hypogonadism was present in 71% of children in group A and 43% of children in group B (p = 0.09).

Hair and skin hypopigmentation was rare in both groups, 38% of patients with microdeletion and 29% of those without the microdeletion (p = 0.56). A common feature of all patients from the group B, compared to 81% from group A (p = 0.02) was small hands and feet. Although a minor diagnostic criteria, it is an important phenotypic feature that may raises clinical suspicion of PWS in the absence of other characteristic features. More than half of patients with microdeletion (52%) and the majority of those without microdeletion (93%) had viscous saliva and angular cheilitis (p = 0.01). The frequency of ocular abnormalities, esotropia and myopia, was about as common in the two groups (52% *versus* 43%, p = 0.58). Language disorders appeared in all patients from the second group compared with 89% from the first one (p=0.23). Patients in both groups had sleep disorders (83% *vs*. 75%, p = 0.57). We diagnosed obstructive apneas (OA), central (CA) and mixed apneas (MA) and hypopneas (hyp); patients associated restless sleep, oral breathing and increased sleepiness during the day. Figure 2 presents the results from one included patient.

Group B included a particular patient; a girl aged 5 years and 5 months, diagnosed with PWS in the first year of life. She had a negative

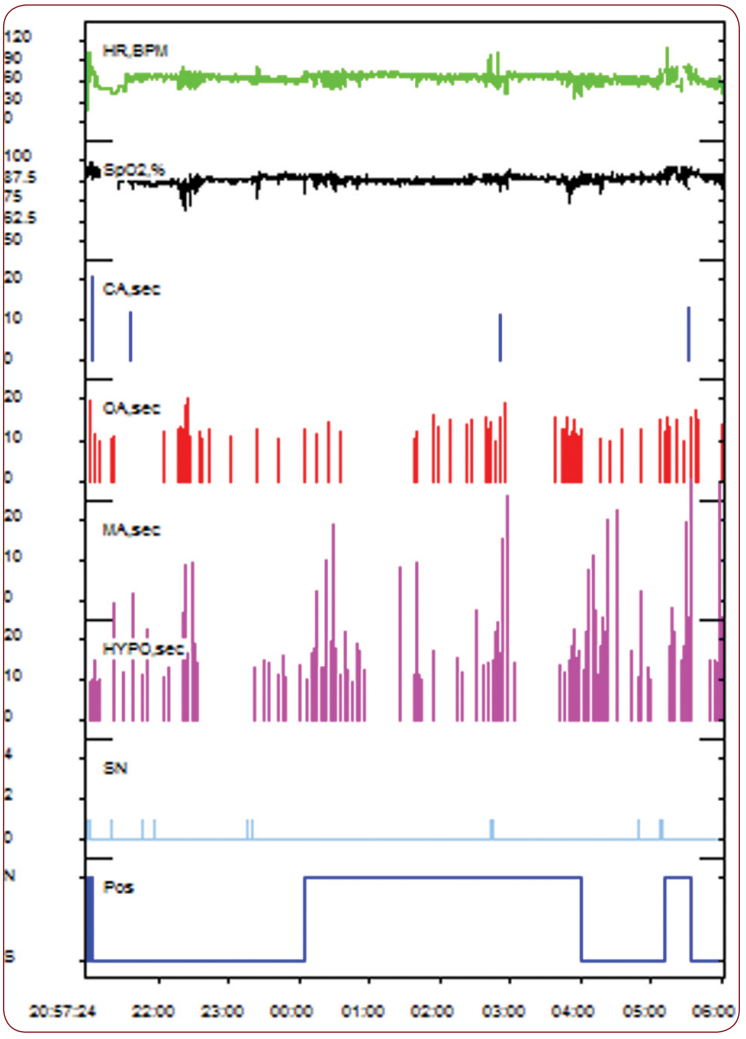

FIGURE 2. Polisomnography- recorded time 546 minutes. We identified 4 central apneas, with duration between 14.9-21.5 seconds, 76 OA (13.7-21 seconds duration) and 149 hyp (19.4-59.5 seconds), the maximum oxygen desaturation was 20 %.

FISH test but a positive methylation analysis; she had an early restrictive diet and medical supervision. The result is a normal weight without an excessive appetite or behavioral problems. ❑

DISCUSSION

The present study evaluated the phenotypic features in a group of patients with PWS examining the correlations with the genotype. We found a higher frequency of major criteria in group A. Facial dysmorphism was diagnosed in all patients in this group, regardless of age. Although the differences are not statistically significant between the two groups, similar results were reported in other studies, indicating an increased incidence of PWS characteristic facial traits in patients with specific microdeletion (10, 11). Several studies reported hypogonadism to be more common in patients with 15q11-q13 region deletion but it was not associated with infertility all the time (12); we didn't identify statistically significant differences between the evaluated groups. Compared with common obesity, patients with PWS show a decrease muscle mass in all regions of the body and an increase adipose tissue (13, 14). Growth hormone (GH) treatment associated with medical supervision and restrictive diet and nutrition improves the ratio between those two and can prevent the obesity (15). Several studies mention a high frequency of neonatal hypotonia in the group without 15q11-q13 region deletion (12). All the evaluated patients of our study had this clinical sign. Hair and skin hypopigmentation was more frequent in the group A, although the differences between the two groups are not statistically significant ($p = 0.56$); this is also suggested by other studies (15, 16). Sleep disorders was observed in both groups, with no statistically significant difference (83% of patients from group A and 75% from group B). They occur due to repeated collapse of the upper airway during sleep, leading to marked reduction (hypopnea) or absent (apnea) airflow. Patient respiratory effort is progressively higher to restore the air flow (17, 18).

Scoliosis is a supportive criterion used to support the clinical diagnosis of PWS. It occurred in the majority of evaluated patients. Spinal abnormalities are exacerbated by excessive weight and require specialized medical therapy (19, 20).

Most of the patient had a late diagnosis a possible explanation could be the fact that PWS is a rare disease, the diagnosis tests are not easily accessible and the medical expertise in different regions is lacking. This could be the reason why some patients did not have the chance to improve the quality of their life. They developed morbid obesity, the PWS characteristic feature, with its associated complications (heart diseases, respiratory and metabolic diseases), without access to recombinant human growth hormone or any other therapy. We also identified some cases where the parents did not want to accept the diagnosis and were reluctant to start a restrictive diet and other adjuvant therapies soon after diagnosis. The literature mentions very good guidelines for PWS patients that should be accessible, known and used by every health provider (21-23).

We did not include in the study the patients that had negative genetic test even if they had a positive clinical score for PWS. They were considered as PWS-like syndrome (24), a group of diseases characterized by PWS phenotype and a different or unknown gene mutation. The literature mentions several genetic abnormalities that could cause PWS-like syndrome. In 2008, Gabett et all reported a duplication of Xq as cause (25, 26); in 2010, 1p36 monosomy (25) or 6p deletion (27). X fragile is also mentioned as cause for PWS like phenotype (24, 27, 28). Other reported causes were 10q26 deletion, 12q subtelomere deletions, X- chromosome abnormalities and Angelman syndrome chromosomal abnormalities in a review performed by Rocha et all (24). Those patients need more advanced genetic tests to establish an etiological diagnosis. Even if they have similar clinical features with PWS, the treatment could be different according to their diagnosis.

Understanding the influence of genotype over the particular phenotype of PWS, especially on behavioral and cognitive characteristics is an early stage of research.

PWS is a rare disease and is difficult to have a larger group of patients to obtain more specific results. It is absolutely necessary to have a specialized center which monitors all PWS patients, which offers a better care for these patients.

Strengths and limitations. One of the main features of presented study is the high cohort. PWS is a rare disease and the total affected patients around the world are reduced. Unfortunately, we do not have a national registry for a better evidence of cases. The good collaboration with Romanian Prader Willi Association, we have the chance to enroll a large number of patients with PWS. We identified the repercussions of a delayed diagnosis and the importance of an easier accessibility to genetic counseling. We also mentioned the presence of Prader Willi-like syndrome among Romanian population, having the aim to open new research areas to identify the genetic substrate of those cases.

We would need more patients information about personal and family medical history to formulate more comprehensive and specific conclusions. The provenience of patients could be an important predictive factor for the early diagnosis because the persons who live in rural areas have a reduced access in medical services. ❑

CONCLUSION

In our study, the various genotypes (the microdeletion or uniparental disomy as genetic causes) did not seem to explain the difference in phenotype in Prader-Willi Syndrome patients. An important finding was the delayed time of diagnosis in these patients, although all had neonatal hypotonia and other suggestive phenotypic features, underlining once more the need for increased awareness of this syndrome amongst neonatologists and pediatricians in Romania and as well as easier accessibility to genetic counseling. Additional research is needed to better understand the genotype-phenotype correlations. ❑

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