Prader-Willy syndrome: heterogeneous genetic mechanisms in a wide phenotypic spectrum

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ABSTRACT

Prader-Willi syndrome (PWS) is an imprinted neuro-behavioral syndrome, affecting many organ systems, characterized by severe hypothalamic-pituitary dysfunction, severe hypotonia, feeding difficulties in the neonatal period, followed by hyperphagia with gradual development of morbid obesity since early childhood, short stature, hypogonadism, infertility, characteristic facial features, impaired motor and language development, cognitive impairment of varying degrees, delayed speech, increased risk of developing autism spectrum disorders (ASD), impaired social skills, and behavioral problems and/or severe psychiatric problems. Loss of expression of the preferentially paternally expressed of genes from the chromosomal region 15q11-q13 are the basis of pathogenesis of PWS, which occur through several mechanisms: deletion of 5-6 Mb DNA fragment from the 15q11-q13 region of the inherited paternal chromosome (65-75%), maternal uniparental disomy (mUPD) (in 20-30%) and Imprinting center (IC) defects, as microdeletions and epimutations (in 1-4%).

In this paper, we present two cases diagnosed with PWS from our experience. The importance of diagnosis and familial recurrence risk will be discussed, as well as genotype-phenotype relationships in PWS. Patients with PWS should benefit from multidisciplinary management very early for greatly improving their quality of life, taking into consideration that obesity is a major factor influencing morbidity and mortality. In addition, a better understanding of the molecular basis of PWS pathogenesis offers hope for the development of new, revolutionary, epigenetic therapies in PWS, as well as in other genetic imprinting diseases.

Keywords: Prader-Willi syndrome, recurrence risk, microdeletion 15q11q13, imprinting center IC

INTRODUCTION

Prader-Willi syndrome (PWS, OMIM #176270, ORPHA:739) is an imprinted neuro-behavioral syndrome, affecting many organ systems, with an estimated prevalence at birth at 1/15,000-30,000 worldwide. PWS is characterized by severe hypothalamic-pituitary dysfunction, severe hypotonia, feeding difficulties in the neonatal period, followed by hyperphagia which causes excessive weight gain with gradual development of morbid obesity since early childhood (even from the age of 3 years) and at adulthood, with an increased risk of developing type II diabetes, short stature associated with growth hormone deficiency. In addition, PWS is characterized by impaired motor and language development, cognitive impairment of varying degrees (mild to moderate), delayed speech, increased risk of developing autism spectrum disorders (ASD), impaired social skills, and behavioral problems and/or severe psychiatric problems. The behavioral phenotype in PWS is often characterized by fits of anger, stubbornness, manipulation, and compulsive behaviors. Hypogonadism is manifested as genital hypoplasia, incomplete pubertal development and, in most cases, infertility in both sexes. Characteristic facial features, strabismus and scoliosis are often present in PWS [1,2].

Changes in the chromosomal region 15q11-q13 are the basis of pathogenesis of PWS, but also of Angelman syndrome (AS), two of the most studied examples of human genomic imprinting. Less than 1% of human genes have differential expression pattern depending on the parental origin, those genes being subjected to genomic imprinting (being active on only 1 member of the chromosome pair). A number of epigenetic factors, such as DNA methylation, histone modifications, chromatin conformation, contribute to this phenomenon. This is also the case for some genes located in the 15q11.q13 region; loss of
expression of the preferentially paternally expressed genes from this chromosomal region, among which the bicistronic SNURF-SNRPN gene and the imprinting center (IC), appear to be very important in PWS etiopathogenesis. On the other hand, loss of expression of the preferentially maternally expressed UBE3A gene localized in the same chromosomal region leads to AS [1,3].

Loss of paternal gene expression in PWS can occur through several mechanisms, most commonly by deleting a 5-6 Mb DNA fragment from the 15q11-q13 region of the inherited paternal chromosome (in 65–75% of cases). In most cases, these interstitial deletions encompassing 5–6 Mb are due to the presence of multiple copies of tandemly repeated sequences at the common breakpoints which flank the deleted region and can cause aberrant recombination of the 15q11.2-q13 region during meiosis, which can result in complex rearrangements in this region (deletions causing PWS or AS, duplications, triplications etc) [3].

Maternal uniparental disomy (mUPD) is also responsible for the loss of paternal gene expression in about 20–30%, in which case there are two chromosomes 15 inherited from the mother and none from the father as a result of a maternal non-disjunction event for chromosome 15 during the maternal meiosis and subsequently trisomy rescue [1].

IC defects such as microdeletions and epimutations (incorrect methylation pattern), which can be inherited or acquired, are responsible for PWS in about 1–4% of the patients, which have biparental allele inheritance, but a maternal-only DNA methylation pattern [1,3,4]. The IC has a bipartite structure, with two critical regions: PWS-IC, a 4.3-kb sequence, which includes the SNRPN promoter/exon 1 and AS-IC, an 880-bp sequence, which is located 35 kb upstream of PWS-IC [5]. Both PWS-IC and AS-IC cooperate, in a complex way, in regulating epigenetic status and allele-specific gene expression in 15q11q13 chromosomal region [6].

Genetic testing for PWS can be covered by a wide range of methodologies, from the classic to the most modern ones, which can detect changes responsible for PWS (microdeletions/ microduplications, uniparental disomy, genomic imprinting) such as: fluorescent in situ hybridization (FISH), quantitative Polymerase Chain Reaction (QF-PCR) analysis, Methyl- ation-Specific Multiplex Ligation-Dependent Probe Amplification (MS-MLPA), Droplet digital PCR, High-resolution SNP microarray, Next Generation Sequencing (NGS) even with the extensive application, Whole-exome sequencing analysis [1,3].

From our experience, we present two cases diagnosed with PWS. The importance of diagnosis and familial recurrence risk will be discussed.

THE FIRST PATIENT: CASE PRESENTATION AND DISCUSSION

The first case presentation is about a newborn male, the second child of a young family, mother 24 years old and the father 31 years old, a 2 years older healthy sister. The pregnancy was unmonitored, with spontaneous delivery. The child had intrauterine growth restriction (IUGR), birth weight 2450g, 50 cm length and APGAR score 9. Even from the neonatal evaluation, important hypotonia was noted. More than that, dysmorphic features, with almond shaped palpebral fissures, micronathia, low set ears, blonde hair, skin hypo-pigmentation, strabismus, high arched palate and feeding difficulties were associated. In this context, the clinical suspicion of a genetic syndrome was raised. Differential diagnosis included spinal muscular atrophy, CNS anomalies, some metabolic inborn errors, Angelman syndrome, fragile X syndrome.

The genetic testing recommended included the first tier blood karyotype, ideally microarray analysis. In this case the blood karyotype had normal result and microarray analysis was performed after that. The SNP-array analysis identified a submicroscopic deletion of 4.9 Mb in the region 15q11.2-15q13.1: arr[GRch37] 15q11.2q13.1(23632678_28539975)x1 (according to ISCN 2020), that is critical for PWS /AS and correlation with the clinical features led to the establishment of a definite diagnosis as PWS for this patient (Figure 1).

The 4.9 Mb deleted region identified in case of our patient contains the whole cluster of imprinted genes which are under control of an imprinting center, IC (the imprinted domain, fig.1), described to be preferentially or exclusively expressed from the paternal chromosome, namely: MAGEL2, NDN, PWRN1, NPAP1 (previously C15orf2), SNURF-SNRPN and several C/D box small nucleolar RNA (snoRNA) genes, up to UBE3A (fig.1). More than that, the deleted region in our patient included also more genes, non-imprinted, bi-parental expressed, between ATP10A and HERC2 genes (fig.1), as found in the majority of patients with PWS and AS, in both syndromes being involved in the same chromosomal region, except for the different location of the deletion, on the paternal chromosome in PWS and on the maternal chromosome in AS [7]. The deleted chromosomal region detected in our patient falls into type II deletions, localized between breakpoint region 2, BP2 and breakpoint region 3, BP3, regions that are susceptible to non-homologous crossovers because they contain large duplicated sequence stretches of 200-400 kb in size. Approximately 60-70% of deletions detected in PWS patients are framed in type II, and only 30-40% in type I deletions, with breaking points BP1 and BP3 (fig.1). In both deletion types, the recurrence risk is <1% in patients
with \textit{de novo} deletion of 15q11q13 on the paternal chromosome (when parental karyotypes are normal) [7,8].

\textbf{THE SECOND PATIENT: CASE PRESENTATION AND DISCUSSION}

The second patient was a newborn girl as first child of another young family, born at 38 weeks of gestation, from uneventful pregnancy, but emergency caesarian section for oligo-hydramnios. The growth parameters were 2770 g, 52 cm length, APGAR score 8. After delivery, she had difficult respiratory adaptation, and oxygen support was added. She had also episodes of hypocalcemia and hypoglycemia. At clinical examination she had dysmorphic features, with almond shaped palpebral fissures, micrognatia, low set ears, inverted nipples, bilateral club foot with 5-th finger clinodactily, hirsutism, important axial hypotonia with reduced reflexes, the whole clinical picture rising suspicion of a genetic syndrome.

To clarify this suspicion, a MS-MLPA analysis for PWS was done, which revealed two hypermethylated/fully methylated DNA copies of the 15q11-q13 region, confirming the diagnosis of PWS, which may have mUPD or IC modifications/defect as a molecular cause in our patient. Studies on IC molecular defects on PWS reported that 15%–20% of those are caused by a microdeletion in the IC, being associated with the 50% recurrence risk, and the other 70-75% of the cases being caused by epimutations, with less than 1% recurrence risk. All these patients have maternal-only DNA methylation pattern, although they have bi-parental inherited the pair of chromosomes 15 [3,4,9]. Microdeletions at PWS-IC on the paternal allele leading to gene silencing across the entire region, leading to PWS are either inherited (about 50%) from an unaffected father (which inherited an IC deletion on his maternally chromosome 15), or \textit{de novo} (the other 50%), occur during spermatogenesis in the father or after fertilization, on the paternally inherited 15 chromosome [5,10].
In order to identify the molecular defect underlying the PWS molecular etiology and establish familial recurrence risk, chromosomal microarray with single nucleotide polymorphism probes (SNP-Array) analysis was done, with normal result for our patient. Taking into consideration that SNP-Array test can only detect UPD isodisomy (UPiD), not heterodisomy (UPhD), in this context, a rare situation of molecular defect/small mutation in IC or maternal UphD (mUPhD) could explain the previous result and clinical outcome.

Therefore, additional analyzes should be performed for further investigation, such as: microsatellite analysis (MSA) for investigating mUPhD, and DNA sequencing of the IC region (in patient and his father) if mUPhD is invalidated. If a de novo mutation (small mutation/microdeletion below the MLPA and SNP-Array detection threshold) is detected in IC region, the recurrence risk is low, <1%, as well as in the case of mUPhD. On the contrary, if this mutation is inherited, the recurrence risk is high, of 50%. So, here are valid arguments in favor of the need to perform an accurate molecular diagnosis, in order to establish familial risk and prenatal diagnosis testing options [7,8,11].

**GENOTYPE-PHENOTYPE RELATIONSHIPS AND OPPORTUNITIES/POTENTIAL FOR EPIGENETIC THERAPIES IN PRADER-WILLI SYNDROME**

Several studies on possible correlations between genotype and phenotype have shown that there is no exclusive link between certain phenotype manifestations and the type of genetic defect in PWS. However, there were some statistical differences in the frequency and/or severity of some symptoms between the top 2 classes of genetic defects in PWS, deletions and UPD.

Thus, in the case of UPD, postpartum birth is more common, and patients with such a genetic defect are less likely to have the typical characteristic facial appearance, a little higher verbal IQ and milder behavior problems, and clinical manifestations of psychosis and autism spectrum disorders (ASD) are much more commonly detected in these patients, in contrast to those in whom the genetic defect is represented by deletions in the 15q11q13 region. In addition, patients with PWS in whom the genetic defect is represented by IC mutations appear to have a classic PWS phenotype, but also an increased predisposition to psychosis similar to UPD [4,10,12].

The study of causal relationships/genotype-phenotype correlations in PWS could provide a solid basis for the development of revolutionary, epigenetic therapies for this syndrome. Recent studies on both cultured PWS patient-derived fibroblasts and PWS mouse models have shown successful first steps in the development of such epigenetic therapies by identifying small molecules with an inhibitory role of EHMT2/G9a (Euchromatic histone-lysine-N-methyltransferase-2/histone 3 lysine 9 methyltransferase), which led to the expression of SNRPN and SNORD116 genes maternally imprinted on maternally inherited chromosome 15 [13].

Another study, focused on understanding/deciphering the mechanisms of genomic imprinting in PWS-specific induced pluripotent cells (iPSC) cell lines and neurons, reported the identification of a complex of ZNF274 and SETDB1 proteins and possibly other components, which may play an important role in establishing the imprinting pattern of the 15q11q13 region since early embryonic development, in regulation of transcription of paternal and silencing of the maternal 15q11-q13 allele. This complex based on ZNF274 and SETDB1 proteins could become the target of therapeutic interventions in PWS, specifically at the SNORD116 locus, which appears to be particularly important in PWS, to avoid adverse therapeutic effects outside the target region [14].

The results of such studies give hope/represent promises for the possibility of developing future epigenetic therapies in PWS, but also in other imprinting disorders, such as related neurodevelopmental disorders: Angelman (AS), 15q duplication (Dup15q), Kagami-Ogata (KOS14), and Temple (TS14) syndromes [15].

**CONCLUSION**

In conclusion, in the case of both patients with PWS presented above, multidisciplinary approach, including genetic evaluation, allowed a relative precocious neonatal diagnosis, guiding the follow up investigations and monitoring, including appropriate neurological, nutritional, endocrinological, physical therapy. As in their case, all patients with PWS should benefit from multidisciplinary management very early for greatly improving their quality of life, taking into consideration that obesity is a major factor influencing morbidity and mortality [1,16].

In addition, a better understanding of the molecular basis of PWS pathogenesis offers hope for the development of new, revolutionary, epigenetic therapies in PWS, as well as in other genetic imprinting diseases.

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