Genotype-Phenotype Relationships and Endocrine Findings in Prader-Willi Syndrome

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Prader-Willi syndrome (PWS) is a complex imprinting disorder related to genomic errors that inactivate paternally-inherited genes on chromosome 15q11-q13 with severe implications on endocrine, cognitive and neurologic systems, metabolism, and behavior. The absence of expression of one or more genes at the PWS critical region contributes to different phenotypes. There are three molecular mechanisms of occurrence: paternal deletion of the 15q11-q13 region; maternal uniparental disomy 15; or imprinting defects. Although there is a clinical diagnostic consensus criteria, DNA methylation status must be confirmed through genetic testing. The endocrine system can be the most affected in PWS, and growth hormone replacement therapy provides improvement in growth, body composition, and behavioral and physical attributes. A key feature of the syndrome is the hypothalamic dysfunction that may be the basis of several endocrine symptoms. Clinical and molecular complexity in PWS enhances the importance of genetic diagnosis in therapeutic definition and genetic counseling. So far, no single gene mutation has been described to contribute to this genetic disorder or related to any exclusive symptoms. Here we proposed to review individually disrupted genes within the PWS critical region and their reported clinical phenotypes related to the syndrome. While genes such as *MKRN3*, *MAGEL2*, *NDN*, or *SNORD115* do not address the full spectrum of PWS symptoms and are less likely to have causal implications in PWS major clinical signs, *SNORD116* has emerged as a critical, and possibly, a determinant candidate in PWS, in the recent years. Besides that, the understanding of the biology of the PWS SNORD genes is fairly low at the present. These non-coding RNAs exhibit all the hallmarks of RNA methylation guides and can be incorporated into ribonucleoprotein complexes with possible hypothalamic and endocrine functions. Also, DNA conservation between SNORD sequences across placental mammals strongly suggests that they have a functional role as RNA entities on an evolutionary basis. The broad clinical spectrum observed in PWS and the absence of a clear genotype-phenotype specific correlation imply that the numerous genes involved in the syndrome have an additive deleterious effect on different phenotypes when deficiently expressed.

Keywords: Prader-Willi syndrome, genotype, phenotype, endocrine, imprinting, SNORDs

INTRODUCTION

Prader-Willi syndrome (PWS; OMIM 176270) was first described in 1956 by Andrea Prader, Alexis Labhart and Heinrich Willi based on a study of nine children with a common clinical tetrad: short stature, intellectual disability, obesity, and small hands and feet (1, 2). The phenotypic analysis was expanded in the following years and decades, revealing the complexity of the syndrome, affecting endocrine, cognitive and neurologic systems, metabolism, and behavior. PWS was the first human disease to be related to genomic imprinting errors, and also the first one shown to be caused by uniparental disomy (3, 4). This rare genetic disorder has a prevalence of 1 in 10,000–30,000 live births, males and females are affected equally in all ethnic groups (5).

The PWS critical region on chromosome 15q11-q13 is monoallelically expressed by paternally inherited genes, exclusively. The absence of expression of one or more of these genes contributes to different phenotypes of PWS (6, 7) and there are three main mechanisms of occurrence: paternal deletion of the 15q11-q13 region; maternal uniparental disomy 15; or imprinting defects (8–10). On the other hand, in the same region, the loss of expression of the *UBE3A* gene (preferentially maternally expressed) drives to Angelman syndrome, with completely different clinical characteristics. By their common implicated region and mechanisms, both syndromes are considered sister imprinted disorders (11, 12).

Clinical manifestations vary with age, impacting multiple body systems (**Table 1**). Fetal size is usually within the normal range. Compared to unaffected siblings, birth weight and body mass index (BMI) are 15% lower on average. Prenatal hypotonia may cause decreased fetal movement, abnormal fetal position at delivery, and increased incidence of assisted delivery or cesarean section (14, 15).

TABLE 1 | Clinical characteristics and the nutritional phases in PWS.

Median ages	Clinical characteristics
Prenatal—birth	Decreased fetal movements
	Lower birth weight and body mass compared to sibs
0–9 months	Severe hypotonia
	Feeding problems and failure to thrive
9–25 months	Improved feeding and appetite
	Normal growth
	Delayed physical and social milestones
2.1–4.5 years	Weight increasing without appetite increase or excess calories
4.5–8 years	Weight increasing with appetite increase
	Global developmental delay
8 years—adulthood	Hyperphagic, rarely feels satiety
	Mild intellectual disability and behavior problems
	Hypogonadism
Adulthood	Appetite no longer insatiable for some
	Short stature and small hands and feet

Gunay-Aygun et al. (13); Miller et al. (14); Driscoll et al. (5).

Severe hypotonia is a clinical hallmark of PWS, leading to failure to thrive during infancy due to lethargy and poor suck. Other common neonatal findings are decreased movement and spontaneous arousal, weak cry, thick saliva, and poor reflexes (13, 16). Around 9 months of life, eating behavior starts to normalize, and the hypotonic status tends to improve, but mild-to-moderate hypotonia persists throughout life, with reduced muscle mass and tone (9, 17).

Physical and social milestones (as sitting, walking, first words, and reading) are delayed and can be achieved at about double the normal age (18). Most individuals have mild intellectual disability, learning difficulties, and poor academic performance. During early infancy, characteristic behavioral problems are common, such as stubbornness, manipulation, compulsiveness, self-injury, and difficulty with change in routine (5, 19, 20). Another common feature in the syndrome is sleep disruption, related to sleep apnea that impairs the quality and efficiency of sleep, frequently associated with excessive daytime sleepiness, and sedentary behavior with a higher predisposition to obesity (13, 21).

In later childhood, individuals with PWS will reach severe obesity unless food intake is strictly controlled by family and caretakers. The lack of satiety (hypothalamic origin) results in hyperphagia, with obsessive food seeking. In uncontrolled cases, obesity, and its complications are the major causes of morbidity and mortality: respiratory insufficiency, cardiovascular problems, metabolic syndrome, sleep apnea, and type 2 diabetes mellitus (22, 23). Mortality rates range between 1.25 and 3% per year (24, 25). Hyperphagia in PWS is still not fully understood and controlling appetite remains a challenge.

The endocrine system can be the most affected in PWS. Growth hormone (GH) deficiency is present in up to 74% of cases and is associated with short stature, small hands and feet, low motor strength, increased fat mass, and decreased movement and energy expenditure (26, 27). GH replacement therapy has shown positive effects not only on growth and body composition but also on development, behavior, and nocturnal respiratory abnormalities, although a careful respiratory follow up is mandatory during long-term GH administration (28–34). Hypogonadism affects both sexes and is manifested as hypogenitalism, incomplete pubertal development and infertility in most individuals (35). Hypogonadism is thought to have a hypothalamic origin, and subsequent insufficient secretion of pituitary gonadotropins and sexual hormones (testosterone or estrogen) (7, 36, 37). Other endocrine abnormalities include hypothyroidism (20–30%), central adrenal insufficiency (about 5%) and type 2 diabetes (up to 25%) due to obesity complications (24, 38–41).

MOLECULAR GENETICS AND DIAGNOSTIC

The hypothalamic dysfunction observed in PWS may be the basis of several symptoms (such as hypotonia, developmental delay or obesity) that overlaps features of other conditions on clinical grounds, like normal obesity and intellectual disability (42).

Definitive diagnosis requires DNA testing. The PWS region spans ~6 Mb on the long arm of chromosome 15 (**Figure 1**). Within this region, at least 2.5 Mb comprises genes with differential expression depending on parental origin. This locus holds protein-coding genes and several non-coding RNAs, which are believed to be involved in the regulation of alternative splicing, mainly in the brain (10, 16).

The bicistronic gene *SNURF-SNRPN* is central to the PWS region and crucial to understanding the methylation pattern in the syndrome. The CpG island at the 5' end of *SNURF-SNRPN* (encompassing the promoter region, exon 1 and intron 1) is differentially imprinted according to parental origin: the unmethylated paternal allele is expressed while the methylated maternal allele is repressed (43). The PWS imprinting center

(PWS-IC, **Figure 1**) involves the CpG island and exon 1 within the 4.3 Kb smallest region of overlap (44). Furthermore, *SNURF-SNRPN* expression produces a long transcript also including PWS-IC, Six snoRNA genes, *IPW* and *UBE3A* antisense (**Figure 1**), which is hypothesized to repress paternal *UBE3A* (45–48).

Most PWS patients (65–75%) present a 5–6 Mb deletion at 15q11-q13 from the paternal origin (16, 49). There are two proximal breakpoints and a common distal breakpoint (**Figure 1**), these regions are flanked by low copy repeat sequences that predispose to abnormal chromosomal pairing and uneven crossing-over, resulting in errors during meiosis (50, 51). Maternal Uniparental Disomy (mDUP) occurs when both chromosomes 15 are inherited from the mother and accounts
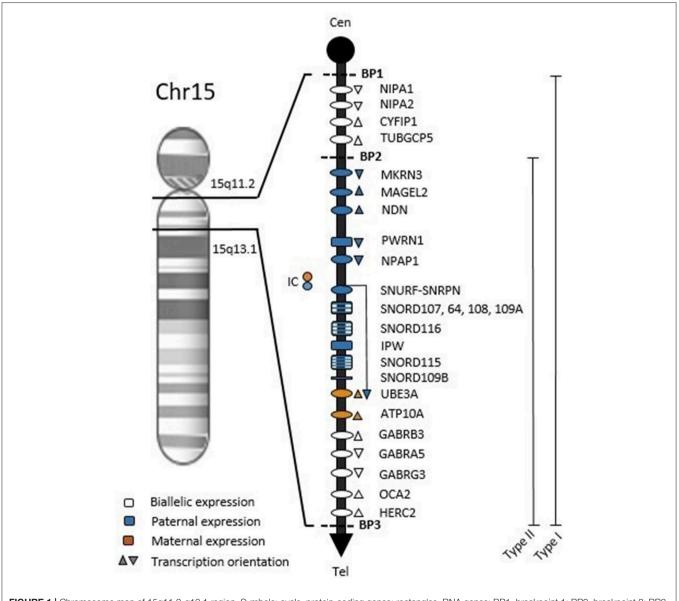

FIGURE 1 | Chromosome map of 15q11.2-q13.1 region. Symbols: ovals, protein-coding genes; rectangles, RNA genes; BP1, breakpoint 1; BP2, breakpoint 2; BP3, breakpoint 3; Type 1, BP1-BP3 deletion with ~6 Mb; Type 2, BP2-BP3 deletion with ~5.3 Mb; Cen, Centromere; Tel, Telomere; IC, Imprinting Center.

for ~20–30% of cases, being associated with advanced maternal age (9, 15). Imprinting defects are caused by epimutations or microdeletions in the PWS-IC in 1–3% of PWS cases. These individuals have biparental allele inheritance, but a maternal-only DNA methylation pattern (11, 52).

Clinical and molecular complexity in PWS enhances the importance of genetic diagnosis in therapeutic definition and genetic counseling. Only DNA methylation analysis can consistently diagnose the syndrome in all three molecular classes (deletion, mUPD, and imprinting defects) and differentiate it from Angelman Syndrome (9, 52). The methylation analysis targets the 5′ CpG island of the *SNURF-SNRPN* locus and will correctly diagnose more than 99% of cases. Currently, there are three assays with this detection capacity: methylation-specific PCR (MS-PCR, the gold standard), methylation-specific multiplex ligation-dependent probe amplification (MS-MLPA) and methylation-sensitive high-resolution melting (MS-HRM) (53–58).

After methylation status confirmation, defining the exact molecular mechanism behind the syndrome origin is important for genetic counseling. Sporadic deletion cases have <1% risk of recurrence, while rare cases of structural abnormalities involving chromosome 15 (such as translocations, ring formation, isochromosome or inversions) can be as high as 25–50% and fluorescence *in situ* hybridization (FISH) can address the deletion source (59–65). mUPD 15 is typically *de novo* (recurrence <1%), proband and parents should be investigated by small nucleotide polymorphisms (SNP) microarray for accurate counseling (66, 67). Most imprinting defects cases are classified as epimutations with no alteration in the DNA sequence and have <1% recurrence risk. However, 15% of individuals with imprinting defects present a paternally inherited microdeletion (7.5–100 kb) in the PWS-IC, in which cases the risk of recurrence is 50%. IC analysis by MS-MLPA or DNA sequencing will address the exact origin of this event (5, 44).

GENOTYPE-PHENOTYPE RELATIONSHIPS IN PRADER-WILLI SYNDROME

None of the PWS genetic errors are associated with exclusive symptoms. However, the most prevalent molecular classes (deletion and mUPD) show statistical differences in frequency or severity in some clinical characteristics. Patients with paternal deletion were more related to feeding problems, sleep disturbances, hypopigmentation and speech and language deficits (68). Individuals with the larger type 1 deletion (**Figure 1**) have been reported to have better academic performance and intellectual abilities, and more compulsiveness when compared to type 2 deletion patients (69, 70). Several other features are more common in mUPD individuals, such as post-term delivery, higher verbal IQ, psychosis and autism spectrum disorder (15, 69, 71–75). On the other hand, mUPD patients are less likely to have the typical PWS facial appearance or hypopigmentation (16). So far, no single gene mutation has been described to contribute to this genetic disorder. Here we proposed to review genes individually disrupted within the PWS critical region and their reported clinical phenotypes related to the syndrome.

The Makorin Ring Finger Protein 3 (*MKRN3*, *ZNF127*) gene encodes a zinc finger protein of the Makorin family and is paternally expressed ubiquitously in human adult tissues, with the highest level in testis, although its exact mechanism of action remains to be elucidated (76). This gene is associated with inhibition of puberty initiation, and loss of function mutations in *MKRN3* are recognized as the main genetic cause of Central Precocious Puberty (77). This correlation has been described by distinct studies with different ethnic groups, affecting equally both sexes, with all mutations segregated in a paternal manner at *MKRN3* (78–81). Experimental models with mice also support the correlation between mutations in *Mkrn3* and puberty dysfunctions, suggesting it may play a role in the hypothalamic-pituitary-gonadal axis (77–79). Altogether, this makes *MKRN3* a strong candidate gene for hypogonadism and infertility in PWS.

The physiological consequence of loss of expression of MAGE Family Member L2 (*MAGEL2*) has been related to phenotypic characteristics of PWS (82). *Magel2*-null mice exhibited endocrine dysfunction similar to PWS: neonatal growth retardation; excessive weight gain; increased adiposity after weaning; impaired hypothalamic regulation and changes in circadian rhythm (83, 84). Hyperphagia, commonly observed in individuals with PWS, is associated with a defect in the hypothalamic arcuate nucleus, which is the major action site of multiple complex interactions between neuropeptide Y (NPY), agouti-related peptide (AgRP), proopiomelanocortin (POMC), and leptin, regulating the food intake and body weight (85, 86). NPY/AgRP interaction stimulates food intake, whereas POMC reduces it. Loss of *MAGEL2* expression disturbs leptin-mediated depolarization of POMC neurons, indicating that food intake is being less repressed and fat storage regulated by leptin is uncontrolled (87, 88). Additionally, loss of expression of *Magel2* impairs reproductive function in mice. *Magel2*-null females showed extended and irregular estrous cycles, while males displayed decreased testosterone levels, and reduced pheromone detection, which has a direct relationship between the main olfactory epithelium and the hypothalamic GnRH neuronal system (89, 90). These results suggest that lack of expression of *MAGEL2* contributes to the reproductive deficiencies observed in PWS and also highlights the role of normal circadian rhythm in maintaining fertility.

Therein, specific point mutations on the paternal allele of *MAGEL2* were reported in 4 individuals with PWS spectrum phenotype: muscle hypotonia, weight gain, developmental delay, and hypogonadism. Although all clinical characteristics were consistent with PWS clinical diagnosis, methylation analysis on the promoter-exon 1 region of the *SNURF-SNRPN* gene showed normal allelic patterns (82). All four subjects were diagnosed with an autism spectrum disorder, intellectual disability, and different degrees of clinical and behavioral features of PWS. Although not a main characteristic, autism is present in 19% of individuals with PWS (71). These four individuals presented a normal methylation pattern, not compatible with PWS, despite similar clinical conditions, which was subsequently called Schaaf-Yang Syndrome (SYS) (91).

Recent data of an international cohort of 78 patients with truncating *MAGEL2* mutations emphasized that SYS overlaps with PWS on clinical grounds in the early stages of life but diverges with the advance of childhood and adolescence (92). PWS features such as hypopigmentation, facial appearance, small hands and feet, thick saliva, behavioral problems are not commonly seen in SYS. And above all distinct symptoms, SYS does not usually cause the high appetite and severe obesity observed in PWS, which can disassociate *MAGEL2* and the hyperphagia condition.

The Necdin (*NDN*) gene encodes a DNA binding protein highly expressed in mature hypothalamic neurons (93). It has been postulated as a key regulator of GnRH levels both *in vitro* and *in vivo*, modulating essential intracellular processes for neurite and axonal outgrowth (94–96). Lack of *NDN* reduces GnRH gene expression, leads to decreased numbers of GnRH neurons, and decreased targeting of GnRH axons to the median eminence of the hypothalamus during development, which can contribute to hypogonadism and infertility in PWS. Also, Necdin paternal-deficient mice were associated with alterations in serotonin and respiratory systems, resulting in irregular breathing and sleep apneas, commonly observed in PWS. Another important evidence reported with *Ndn*-KO mice was sudden death due to respiratory disorders, which is the main side effect associated with GH therapy (97–99). *NDN* might be a genetic factor contributing to apneas and respiratory dysfunctions of PWS.

Interestingly, (100) described three patients with atypical deletions related to PWS. Patient 1 was deleted for *MKRN3*, *MAGEL2*, and *NDN* with no PWS major clinical criteria, except for obesity, developmental delay, and high pain threshold. Patients 2 and 3 had a deletion encompassing *NPAP1*, *SNURF-SNRPN*, and the SNORD genes, but did not reach *MKRN3*, *MAGEL2*, and *NDN*, and presented PWS major clinical signs (100). This report suggests that a paternal deficiency of *MKRN3*, *MAGEL2*, and *NDN* is not sufficient to generate the full PWS phenotype and postulates *NPAP1*, *SNURF-SNRPN*, and the SNORD genes (discussed ahead) to be the critical region for PWS. These results contradict other studies and exemplify the complexity to establish a genotype-phenotype relationship in PWS (78, 82, 83, 98, 99, 101, 102).

The Prader-Willi region encompasses a series of long non-coding RNAs (lncRNAs) which are characteristically more than 200 nucleotides long and can be involved in epigenetic modifications of DNA, and regulation of gene expression at transcriptional and post-transcriptional levels (103–105). The first lncRNA inside the PWS region is the Prader-Willi Region Non-Protein Coding RNA 1 (*PWRN1*), biallelically expressed in the testis and kidneys, and monoallelic expressed in the brain, in addition to being an alternative 5′ part of SNURF–SNRPN (106). Wawrzik et al. (107) hypothesized that the action of *PWRN1* on the imprinting mechanism may be indirect through keeping the paternal allele in an open chromatin configuration, allowing access to transcription factors (107). The main limitation for further confirmation studies is the lack of gene orthology in mice (108–110).

The Nuclear Pore Associated Protein 1 (*NPAP1*), formerly known as Chromosome 15 Open Reading Frame 2 (C15orf2), is an intronless gene that is biallelically expressed in adult testis and monoallelically expressed in fetal brain, including the hypothalamus which is related to several endocrine features of PWS (106, 111). Moreover, this gene is associated with the Nuclear Pore Complex (NPC), in which the main function is to regulate macromolecular transport between the nucleus and the cytoplasm. NPCs also participates in several nuclear processes, such as gene regulation, mRNA biogenesis, and cell cycle control. Likewise *PWRN1*, due to the lack of orthology in mice the exact role of the *NPAP1* gene in the development of PWS is not clear (112).

The Small Nuclear Ribonucleoprotein Polypeptide N (*SNRPN*) gene is located within the central region associated with PWS and has an important regulatory role over the imprinted genes located in chromosome 15 (113, 114), while the SNRPN Upstream Reading Frame (*SNURF*) gene is encoded by an evolutionarily-conserved upstream open reading frame and is localized to the nucleus (115). *SNURF-SNRPN* is a complex bicistronic gene encoding two different proteins, and the PWS-IC is found at its 5′ end. *SNURF* is encoded by exons 1–3 and produces a small nuclear protein of unknown function (113), exons 4–10 correspond to the *SNRPN* portion and encode the protein SmN, involved in mRNA splicing (43). It also holds six snoRNA genes located telomerically which are expressed as a long transcript (46). The SmN protein shows the highest expression in the brain and heart (115–117). Despite its central position in PWS, the function and regulation of the many alternative transcripts of *SNURF-SNRPN* are still poorly understood (48).

Within the long *SNURF-SNRPN* transcript, there are a series of Small Nucleolar RNAs (snoRNAs) thought to participate in DNA methylation, alternative splicing and post-transcriptional regulation (10, 118). The PWS region encompasses five single copy snoRNA genes (*SNORD64*, *SNORD107*, *SNORD108*, *SNORD109A*, and *SNORD109B*) and two snoRNA gene clusters (*SNORD115* and *SNORD116*). The expression of SNORD genes varies in different human and mouse tissues, suggesting specificity in post-transcriptional activity (46, 119–122). Although most of the SNORDs are ubiquitously expressed in human tissues, *SNORD115* and *SNORD109B* appear to be restricted to the brain. Our understanding of the single-copy SNORDs in PWS remains extremely limited, but some progress has been made with the clusters: *SNORD116* has 29 tandemly repeats and *SNORD115* is composed of 48 gene copies (118). Given that SNORD sequences are well-conserved across placental mammals (especially in primates and rodents), this suggests they have an evolutionary functional role (123, 124).

A minimal critical region has emerged implicating that the *SNORD116* cluster is crucial for most of the PWS phenotype, based on clinical evidence on rare patients with small deletions (150–200 Kb) or translocations (11, 125–128). Experimental studies on *Snord116*-KO mice displayed PWS features such as post-natal growth retardation and hyperphagia (129–132). Remarkably, a *Snord116*-KO mice model specifically in NPY neurons in the hypothalamic arcuate nucleus summarized the

same overall phenotype observed in mice lacking *Snord116* globally; low birth weight, increased body weight gain in early adulthood, increased energy expenditure and hyperphagia (130). This suggests an important role of *Snord116* in controlling NPY neuronal functions, and thus food intake and energy homeostasis. Also, a recent study reported *Snord116*-deficient mice with decreased activity of the hypothalamic prohormone convertase PC1 impairing the prohormone processing of proinsulin, pro-GH-releasing hormone, and proghrelin, pointing to an important part of *SNORD116* and PC1 deficiency in the main neuroendocrine features of PWS (133). Interestingly, it was shown that a mouse *Snord116* deletion model displayed loss or shift in methylation dynamics in 97% of CpG islands in the cerebral cortex dependent on the circadian cycle. And this disrupted epigenetic rhythm had a strong overlap between mouse and human genes related to meal timing, circadian biology, and obesity (134). In the recent years, *SNORD116* has emerged as a critical, and possibly, determinant candidate in PWS not only by its highly conserved sequence in the minimal critical region, but also because paternal deletions affecting the expression of *NDN*, *MKRN3*, *MAGEL2*, or *SNORD115* genes do not address the full spectrum of PWS symptoms (10, 100, 123, 135, 136).

The Imprinted in Prader-Willi Syndrome (*IPW*) gene is a lncRNA known to modulate another evolutionarily distinct imprinted gene cluster at the human chromosomal region 14q32 expressed only from maternally inherited alleles (137). *IPW* is widely expressed both in fetal and adult tissues, exclusively from the paternal allele (138). It has been postulated that *IPW* has no biological consequences in PWS, based on the relatively poor conservation between human and mouse sequences (138), and the fact that mice with a paternally inherited deletion including *Ipw* did not show PWS symptoms (139). However, Stelzer et al. (137) proposed that lack of expression of *IPW* results in aberrant upregulation of maternally expressed genes at the 14q32 imprinted cluster, pointing that the action of *IPW* on the imprinting mechanism of this locus occurs by histone modification, and consequently, transcription reduction (137). This hypothesis is supported by clinical reports of affected individuals with mUPD 14 (overexpression of maternal genes) presenting PWS-like phenotypes, such as neonatal hypotonia, small hands and feet, intellectual disability and hyperphagia (140–142). These findings pinpoint a regulatory cross-talk between 15q11-13 and 14q32 imprinted loci, but further, suggest that some PWS phenotypes may arise from different chromosomal regions other than the PWS critical locus (143, 144).

SNORD115 gene is the most characterized SNORD within the PWS region. It presents a complementary sequence of 18 nucleotides with the mRNA encoding the serotonin receptor 5-HT2C, perfectly base pairing with exon V that undergoes both alternative RNA splicing and RNA editing (post-transcriptional changes to specific nucleotide sequences) (118). Mice with a large deletion encompassing the *Snord115* cluster developed normally to adulthood with apparently no significant defects (139). And there are also clinical reports on patients with an entire deletion of the *SNORD115* gene cluster that did not present any PWS major clinical signs

(135, 136). Taken together, these findings suggest that lack of *SNORD115* is not sufficient to cause PWS, but a phenotypic effect when absent along with other genes in the PWS critical region cannot be excluded. Actually, the *5-HT2C* gene encodes G protein-coupled receptor specific to the brain, whose activation is associated with a variety of physiological processes, such as dopamine modulation, anxiety, sleep regulation, satiety response, energy balance, and locomotor activity (145). Interestingly, experimental studies have described 5-HT2C receptor knockout mice that developed are hyperphagia and late-onset obesity, two major clinical features of PWS in humans (146, 147). Therefore, the absence of *SNORD115* expression in PWS accompanied by the possible post-transcriptional impairment of the 5-HT2C receptor activity may be partly responsible for some of the behavioral and metabolic features of the syndrome.

The establishment of a causal genotype-phenotype relationship can bring light to new therapeutic approaches for PWS. Epigenetic therapy has been used in cancer treatment mostly focusing on the identification of small molecules and compounds with the capacity to reverse the epigenetic changes (epigenome reprogramming) (145, 148). The successful experience obtained from the epigenetic-cancer therapies contributes to the development of similar approaches for genomic imprinting disorders. Recent studies have shown that histone methyltransferase inhibitors are capable of reactivating the expression of paternally expressed SNRPN and *SNORD116* from the maternal chromosome, both in PWS mouse models and in cultured PWS patient-derived fibroblasts (149, 150). Although further investigation needs to be performed *in vivo*, epigenetic therapy aiming PWS genes in the maternal chromosome could reverse, or at least regulate, some PWS clinical conditions such as hyperphagia and behavioral problems (151). This data supports future studies to assess translational epigenetic-based therapies for PWS in humans.

CONCLUSION

PWS is a complex imprinting disorder caused by the lack of expression of paternally-inherited genes on chromosome 15q11-q13 with severe implications on endocrine, cognitive and neurologic systems, metabolism, and behavior. The PWS critical region encompasses five protein-coding genes (*MKRN3*, *MAGEL2*, *NDN*, *NPAP1*, and *SNURF-SNRPN*) and more than 80 RNA genes (*PWRN1*, *IPW*, and several SNORDs) but their contribution to unique PWS phenotypes is still unclear. The broad clinical spectrum and the absence of a clear genotype-phenotype specific correlation imply that the numerous genes involved in PWS have an additive deleterious effect when deficiently expressed. So far, the lack of expression of the *SNORD116* gene cluster has arisen as the best explanation for most of the PWS phenotype, yet there is a clear need to investigate more of its mechanism of action, especially the incorporation into ribonucleoprotein complexes, possibly acting in hypothalamic and endocrine functions in adulthood and perinatal period. Besides *SNORD115* and *SNORD116*, our understanding of the

biology of the PWS SNORD genes is still rather shallow. These SNORDs exhibit all the hallmarks of RNA methylation guides and can associate with other proteins to form functional ribonucleoprotein complexes. Also, the SNORD sequences are well-conserved across placental mammals, strongly asserting that they have a functional role as RNA entities under evolutionary pressure. A better understanding about genotype-phenotype in PWS can open space for new therapeutic approaches especially for patients that present side effects related to the current standard treatment, and develop genetic counseling for the different levels of severity in PWS that require specific and constant medical follow-up, improving the life quality of patients, family, and caretakers.

AUTHOR CONTRIBUTIONS

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