



# Genetics of Prader-Willi syndrome and Prader-Will-Like syndrome

Chong Kun Cheon, MD, PhD

Division of Pediatric Endocrinology and Metabolism, Department of Pediatrics, Pusan National University Children's Hospital, Pusan National University School of Medicine, Yangsan, Korea

The Prader-Willi syndrome (PWS) is a human imprinting disorder resulting from genomic alterations that inactivate imprinted, paternally expressed genes in human chromosome region 15q11-q13. This genetic condition appears to be a contiguous gene syndrome caused by the loss of at least 2 of a number of genes expressed exclusively from the paternal allele, including *SNRPN*, *MKRN3*, *MAGEL2*, *NDN* and several *snoRNAs*, but it is not yet well known which specific genes in this region are associated with this syndrome. Prader-Will-Like syndrome (PWLS) share features of the PWS phenotype and the gene functions disrupted in PWLS are likely to lie in genetic pathways that are important for the development of PWS phenotype. However, the genetic basis of these rare disorders differs and the absence of a correct diagnosis may worsen the prognosis of these individuals due to the endocrine-metabolic malfunctioning associated with the PWS. Therefore, clinicians face a challenge in determining when to request the specific molecular test used to identify patients with classical PWS because the signs and symptoms of PWS are common to other syndromes such as PWLS. This review aims to provide an overview of current knowledge relating to the genetics of PWS and PWLS, with an emphasis on identification of patients that may benefit from further investigation and genetic screening.

**Keywords:** Prader-Willi syndrome, Prader-Willi-like syndrome, Imprinting disorder, Genetic screening

## Introduction

Prader-Willi syndrome (PWS; OMIM #176270) is an imprinted neurobehavioral condition affecting many organ systems and occurs due to the absence of expression of a cluster of paternally expressed genes located at 15q11-q13<sup>1)</sup>. PWS is characterized by 2 phases in terms of clinical features. In infancy there is a failure to thrive, muscular hypotonia, genital hypoplasia, respiratory problems, and feeding difficulties<sup>2)</sup>. From as early as 2 years of age, an altered phenotype becomes apparent with evidence of mild developmental delay and learning disabilities and the onset of severe overeating behavior resulting from an abnormal satiety response to food intake<sup>3)</sup>. Other later-phase phenotypic characteristics include growth hormone deficiency, short stature, small hands and feet and significant behavioral problems<sup>1,2)</sup>. Recent epidemiological study estimates an incidence of 1 in 25,000 births and a population prevalence of 1 in 50,000<sup>4)</sup>. Paternally expressed genes are particularly important in hypothalamic development, as indicated by the hypothalamic accumulation of androgenetic (duplicated paternal genome) cells in chimeric mouse embryos<sup>5,6)</sup>. Paternal *de novo* deletions of the 15q-q13 region account for about 70% of PWS. Most of the remaining cases have uniparental maternal disomy (UPD) for chromosome 15. The PWS region includes a few protein-coding genes and multiple paternally expressed noncoding RNAs, several of which were previously suggested to regulate alternative splicing<sup>7,8)</sup>. Several imprinted genes

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### Address for correspondence:

Chong Kun Cheon, MD, PhD  
Division of Pediatric Endocrinology and Metabolism, Department of Pediatrics, Pusan National University Children's Hospital, Pusan National University School of Medicine, 20 Geumo-ro, Mulgeum-eup, Yangsan 50612, Korea  
Tel: +82-55-360-3158  
Fax: +82-55-360-2181  
E-mail: chongkun@pusan.ac.kr

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or transcripts have been mapped to 15q11-q13, most with only paternal expression, including *SNURF-SNRPN*, several clusters of small nucleolar RNAs (snoRNAs), *NDN*, *MKRN3*, *NPAP1*, and *MAGEL2*<sup>9</sup>). The noncoding RNAs are highly expressed in the brain and function through modification of ribosomal RNAs<sup>10</sup>. Nevertheless, the functions of the vast majority of genes residing in the PWS region remain to be determined. The syndrome has a clinical overlap with other diseases, which makes it difficult to accurately diagnose. The challenge for clinicians is not only to differentiate more clearly between PWS and the various Prader-Will-Like syndrome (PWLS) on a clinical level but also to provide conclusive genetic explanations for these phenotypes to provide accurate genetic counseling and treatment. The absence of a correct diagnosis may worsen the prognosis of these individuals due to the endocrine-metabolic malfunctioning associated with the PWS. Therefore, an accurate chromosomal investigation is necessary to differentiate classical PWS from the PWLS. This review aims to provide an overview of current knowledge relating to the genetics of PWS and PWLS.

## Molecular and genetic basis of PWS

### 1. Structure and genes in the 15q11-q13 region

The 15q11.2-q13 region can be roughly divided into 4 distinct regions that are delineated by 3 common deletion breakpoints<sup>11</sup>, which lie within segmental duplications<sup>12</sup> (Fig. 1). First, proximal nonimprinted region between the 2 common proximal breakpoints (BP I and BP II) containing four bi-parentally expressed genes, *NIPA1*, *NIPA2*, *CYFIP1*, and *TUBGCP5*<sup>13</sup>. Second, the PWS domain contains five paternally expressed protein-coding genes (*MKRN3*, *MAGEL2*, *NDN*, *snoRNAs*, and *SNRPN-SNURF*, *C15orf2*) and several antisense transcripts (including the antisense transcript to *UBE3A*)<sup>9,14</sup>. Third, the Angelman syndrome (AS) domain containing the

preferentially maternally expressed genes (MEGs) (*ATP10A* and *UBE3*). Fourth, a distal nonimprinted region containing a cluster of three gamma-aminobutyric acid receptor genes, the gene for oculocutaneous albinism type 2 (*OCA2*), *HERC2*, and the common distal breakpoint (BP III).

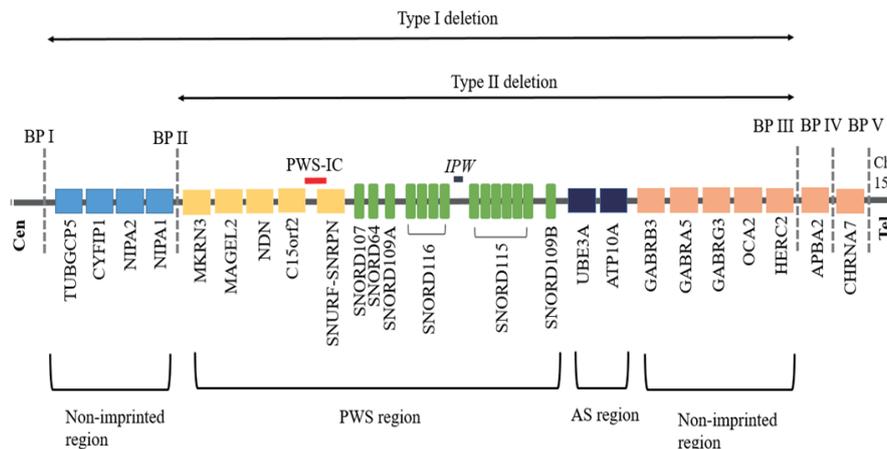
The genomic and epigenetic changes causing PWS all lead to a loss of expression of the normally paternally expressed genes on chromosome 15q11.2-q13<sup>15</sup>. Absence of the paternally inherited copy of these genes, or failure to express them, causes total absence of expression for those genes in the affected individual because the maternal contribution for these genes has been programmed by epigenetic factors to be silenced<sup>16</sup>. The 15q11.2-q13 region is highly vulnerable to structural rearrangements, such as deletions, duplications, supernumerary marker chromosomes, and translocations due to presence of low-copy repeats (LCRs) in the region<sup>17,18</sup>. The exact function of each of the genes in determining the PWS phenotype remains to be elucidated, although possible insight has been gained by work with mouse models by multiple investigators<sup>15</sup>.

### 2. Allelic variants related to PWS

PWS is a contiguous gene disorder, as studies thus far indicate that the complete phenotype is due to the loss of expression of several genes<sup>15</sup>. The search for candidate genes contributing to specific phenotypic components of PWS has been extensively performed.

#### 1) *SNURF-SNRPN* gene

Central to the PWS region is the *SNURF-SNRPN* gene which is an extremely complex gene locus that spans, 465 kb, with 148 possible exons that undergo alternative splicing<sup>4,19</sup>. It is a bi-cistronic gene encoding two different proteins<sup>15</sup>. Exons 4–10 were described first and encode the protein SmN, which is a spliceosomal protein involved in mRNA splicing<sup>20</sup>. *SNURF* is encoded by exons 1–3, which produces a polypeptide of



**Fig. 1.** Ideogram of chromosome 15q11-q13 showing genes located in the typical deletion region of Prader-Willi syndrome. BP, breakpoint; PWS-IC, Prader-Willi syndrome-imprinting center; AS, Angelman syndrome.

unknown function<sup>21</sup>). At the 5' end of the *SNURF-SNRPN* gene is a CpG island encompassing the promoter, exon 1, and intron 1<sup>15</sup>). Imprinting occurs partly through parent-of-origin allele-specific methylation of CpG residues, which is established either during or after gametogenesis and maintained throughout embryogenesis<sup>22</sup>). This is a differentially methylated region, which is unmethylated on the paternally inherited expressed allele and methylated on the maternally inherited repressed allele<sup>20</sup>). The *SNRPN* minimal promoter region includes 71 bp of upstream sequence and the first 51 bp of *SNURF-SNRPN* exon 1<sup>23,24</sup>) and the integrity of the *SNRPN* minimal promoter region/exon1 region appears to be essential part of the imprinting center (IC) in the PWS chromosomal region in both mice and humans, and upstream sequences are critical for the correct function of either mechanism<sup>25,26</sup>). The *SNURF-SNRPN* gene also serves as the host for the six *snoRNA* genes which are regulated by the expression of *SNURF-SNRPN* and do not encode proteins<sup>19,27,28</sup>). Changes in the noncoding regions can cause genetic disease by altering gene expression. Wu et al.<sup>29</sup>) showed that mutations upstream of *SNRPN*/exon1 caused lack of methylation in the maternal *SNRPN* promoter and activation of MEGs including a rescue from the lethality and growth retardation normally shown by the PWS mouse. Maina et al.<sup>23</sup>) suggested that changes in the maternal *SNRPN* minimal promoter region may ameliorate some of the more severe symptoms of the disease shown by 9 PWS patients with atypical genetics.

## 2) *snoRNA* genes

The *snoRNA* genes located in the large *SNURF-SNRPN* transcripts present not only in single copy (*SNORD64*, *SNORD107*, *SNORD108*, *SNORD109A*, and *SNORD109B*) and but also in the two *snoRNA* gene clusters (*SNORD115* and *SNORD116*)<sup>9,15</sup>). It is thought that the *snoRNAs* may target cellular mRNAs for methylation or alternative splicing because the *snoRNAs* in the PWS region lack the usual rRNA complementarity and that each *snoRNA* gene might have multiple targets<sup>19,27</sup>). The *snoRNA* gene may be responsible for at least several features of PWS on the basis of 6 patients with balanced translocations affecting the *SNURF-SNRPN* locus, who were described to have typical PWS or a PWLS phenotype<sup>30-32</sup>). A "key" region to explain much of the PWS phenotype has been narrowed to the *SNORD116* *snoRNA* gene cluster<sup>33</sup>). Mice lacking the *SNORD116* orthologue display a partial PWS phenotype<sup>34</sup>). The *SNORD116* has been shown to be highly conserved in rodents, and Gallagher et al.<sup>28</sup>) showed that a 121-kb region thought to be critical in PWS contained both *SNORD116* and *SNORD109*, suggesting that these genes may play a major role in the PWS phenotype. Up to date, most reported clinical cases of limited deletion of the *SNORD116* cluster associated with PWS have also involved adjacent genes: *SNURF-SNRPN* or *SNORD115*<sup>34,35</sup>). But, a crucial role for the *SNORD115* locus was eliminated by an AS family with a familial microdeletion that included the entire *SNORD115* gene cluster and the *UBE3A* locus<sup>36</sup>). There have been three separate reports of three different individuals with overlapping microdeletions

(175–236 kb) that all encompass the *SNORD116* gene cluster<sup>34,35</sup>). All three have multiple clinical features typical of PWS including neonatal hypotonia, infantile feeding problems, rapid weight gain by 2 years of age, hyperphagia, hypogonadism, developmental delay/intellectual disability, and speech and behavioral problems<sup>15</sup>). However, these three individuals also have features not typical of classical PWS, including tall stature, macrocephaly, lack of a "PWS facial gestalt," and atypical hand features of PWS. More recently, Bieth et al.<sup>37</sup>) reported the first case of a patient with the highly typical features of PWS who presented a restricted deletion of the *SNORD116* region which did not affect the expression of *SNURF-SNRPN* and did not delete any portion of the *SNORD115* locus. This finding in a human case might suggest that a lack of the paternal *SNORD116* gene cluster has a determinant role in the pathogenesis of PWS.

## 3) *MAGEL2* gene

The *MAGEL2* is located adjacent to *NDN* in the human and mouse, with highest expression in mouse at late developmental stages and in the hypothalamus and other brain regions and considered to be a candidate gene for the eating disorder of PWS<sup>38-40</sup>). Wevrick et al.<sup>41-43</sup>) have reported that *Magel2*-null mice have selected biological findings similar to PWS in humans, including neonatal growth retardation, excessive weight gain after weaning, impaired hypothalamic regulation and reduced fertility. Recently, Schaaf et al.<sup>44</sup>) reported 2 patients with point mutations in the imprinted *MAGEL2* gene in the 15q11-q13 domain causing classic PWS, suggesting that that *MAGEL2* loss of function can contribute to several aspects of the PWS phenotype.

## 4) *NDN* gene

Among the imprinted candidate genes for PWS, the gene *NDN* encoding the MAGE family NECDIN protein proposed to act as a neuronal growth suppressor and antiapoptotic protein in postmitotic<sup>44</sup>). A mouse *Ndn* knockout model has been reported with similar defects to individuals with PWS<sup>45</sup>) and mouse *Ndn* mRNA is expressed predominantly in a subset of postmitotic neurons, with highest levels in the hypothalamus and several other brain regions at late embryonic and early postnatal stages, as well as other tissues<sup>46</sup>).

## 5) *MKRN3* gene

The *MKRN3* encodes the makorin ring finger protein 3 and the *MKRN3* differential allele expression occurs through silencing of the *MKRN3* maternal allele, which is associated with 5' CpG island methylation<sup>47</sup>). The functional and physiological relevance of *MKRN3* is not well known and despite its location in the PWS critical region, its role in this syndrome is also unclear. Recently, it was investigated if central precocious puberty (CPP) could arise from loss of *MKRN3* expression by the paternal allele due to a *de novo* deletion, maternal UPD or an imprinting defect, mechanisms recognized in the pathogenesis of the PWS<sup>48</sup>). Particularly, a girl with a paternal deletion in *MKRN3*, *MAGEL2* and *NDN* genes, who had few features of

the PWS, was diagnosed as CPP<sup>49</sup>). In addition, few cases with the PWS and CPP have been reported<sup>50</sup>). Potentially, human genomic sequence and global methylation analyses in PWS patients with CPP could establish epigenetic alterations in the pathogenesis of the disorder.

#### 6) *IPW*

Many long noncoding RNAs (lncRNAs) interact with chromatin-modifying proteins owing to their secondary structure and can recruit chromatin-modifying complexes to specific genomic regions. *IPW* located within the critical PWS-associated region on chromosome 15 is one of several lncRNAs associated with an imprinted locus, which is considered to be an RNA transcript only, because it does not encode a protein<sup>1</sup>). Stelzer et al.<sup>51</sup>) demonstrate that a paternally expressed lncRNA known as *IPW* has a role in modulating the expression of MEGs and identified a critical role for *IPW* in modulating the expression of MEGs *in trans*, which has important implications for the understanding of imprinted gene networks. Loss of imprinted genes in the PWS locus thus leads to an effect in *trans* of increased expression of imprinted genes in the *DLK1-DIO3* locus on chromosome 14, suggesting that there might be cross-talk between imprinted loci.

#### 7) Nonimprinted genes (*NIPAI*, *NIPA2*, *CYFIPI*, and *GCP5*)

It is unclear whether the four, nonimprinted genes (*NIPAI*, *NIPA2*, *CYFIPI*, and *GCP5*) localized to the interval between BP I and BP II, contribute towards the PWS phenotype<sup>44</sup>).

### 3. Molecular classes of PWS

There are 3 main classes of chromosomal abnormalities that lead to PWS: deletion on 15q11-q13, maternal UPD of chromosome 15, or a defect in the IC on 15q11-q13, although gene mutation (<0.1%) and balanced translocation (0.1%) can also be found<sup>52,53</sup>).

#### 1) Microdeletion of the chromosome region 15q11-q13 deletion

Most patients with PWS result from an interstitial microdeletion of the paternally inherited 15q11.2-q13 region. Deletions occur in about 65%–75% of the patients with PWS and AS. Deletions in PWS and AS are subdivided into 2 main subgroups (types I and II) and the BPs are flanked by LCRs in the 3 BPs<sup>12</sup>). Two common classes of deletions of the PWS/AS critical region have been described; type I (40%), approximately 6 Mb in size between BP I and BP III and type II (60%), spanning 5.3 Mb between BP II and BP III<sup>54</sup>). Individuals with deletion of type I show a more severe phenotype than type II<sup>11,12,55</sup>). Both types I and II deletions are almost always *de novo* events<sup>56</sup>). These recurrent common interstitial deletions measure approximately 5–6 Mb in size and are due to the presence of multiple copies of tandemly repeated sequences at the common breakpoints (BP I, BP II, and BP III) flanking the deleted region<sup>15</sup>). These LCRs sequences stretch for approximately 250–400 kb and can

cause nonhomologous pairing and aberrant recombination of the 15q11.2-q13 region during meiosis, leading to deletions, duplications, triplications, and inverted dup (15)<sup>57</sup>). In addition, approximately 8% of those with a deletion have a unique or atypical sized deletion (i.e., not type I or II) from a variety of etiologies, including an unbalanced translocation<sup>56</sup>). A deletion that is smaller or larger than typically seen in PWS may affect the phenotype rare and have been important for the delineation of genotype-phenotype correlation<sup>9</sup>).

#### 2) Uniparental disomy of chromosome 15

Maternal UPD 15 is the situation in which there are 2 chromosomes 15 from the mother and none from the father<sup>58</sup>). This accounts for approximately 20%–30% of individuals with PWS. Maternal UPD has been shown to be associated with advanced maternal age<sup>59,60</sup>). Trisomy associated with Robertsonian translocations may resolve to disomy through loss of a chromosome and would result in UPD in 50% of cases<sup>15</sup>). UPD can be associated with small supernumerary chromosome 15 markers, and both maternal and paternal UPD 15 have been identified from this situation, although maternal is more common<sup>61</sup>). The parental origin of these small markers is frequently unknown due to the small size and lack of unique genetic material. It has been estimated that approximately 5% of small supernumerary markers are associated with UPD<sup>62</sup>).

#### 3) Imprinting defect

This molecular class affects the imprinting process on the paternally inherited chromosome 15 and accounts for approximately 1%–3% of individuals with PWS<sup>15</sup>). Most IDs result from epigenetic causes (epimutations) and demonstrate a maternal-only DNA methylation pattern despite the presence of both parental alleles (i.e., biparental inheritance)<sup>15</sup>). DNA sequence changes are not found in these epimutations, and they are thought to be random errors in the imprinting process or in early embryogenesis in the rare cases of somatic mosaicism<sup>16</sup>). However, approximately 15% of individuals with an ID are found to have a very small deletion (7.5 to >100 kb) in the PWS IC region located at the 5' end of the *SNRPN* gene and promoter (i.e., IC deletion)<sup>63</sup>). Of these, about half have been inherited from an unaffected father with the IC deletion on his maternally inherited chromosome 15<sup>15</sup>). The other half are *de novo* IC deletions on the paternally inherited 15 that occur during spermatogenesis in the father or after fertilization<sup>59,64</sup>).

### Genotype-phenotype correlations

There are no features known to occur exclusively in individuals with one of the genetic classes<sup>15</sup>). However, there are some statistical differences in the frequency or severity of some features between the 2 largest classes (deletion 15q11.2-q13 and UPD)<sup>15</sup>). Postterm delivery is more common with UPD<sup>65</sup>). Individuals with UPD are less likely to have the typical characteristic facial appearance<sup>59,60</sup>), or skill with jigsaw

puzzles<sup>66</sup>). Patients with deletions have a higher frequency of hypopigmentation of skin, hair and eyes due to loss of expression of the nonimprinted *P* gene that is involved in oculocutaneous albinism<sup>59,60</sup>. In most studies, those with UPD have a little higher verbal IQ and milder behavior problems<sup>67</sup>. Interestingly, psychosis and autism spectrum disorders (ASD) are almost shown to PWS adults with UPD rather than deletions<sup>68</sup>. Recent studies suggest that as many as 62% of those with UPD develop atypical psychosis compared with 16% of those with a deletion<sup>69</sup>. PWS subjects with IC mutations appear to have a classical PWS phenotype and might have a similar increased predisposition to psychosis as UPD<sup>70</sup>. Torrado et al.<sup>71</sup> reported that patients with a deletion type had a higher frequency of need for special feeding techniques, sleep disturbance, hypopigmentation, and speech articulation defects. Several studies have investigated phenotypic characteristics between PWS individuals with type I versus type II deletions, but there has been a lack of consensus among the different studies. For example, Butler et al.<sup>72</sup> reported 12 patients with type I deletion showed worse adaptive behavior, more severe compulsive behavior and more impairments in reading, math skills and visual perception than 14 patients with type II deletion. On the other hands, Milner et al.<sup>73</sup> did not find any significant phenotypic differences between the 2 main deletion subtypes (type I, n=14; type II, n=32). As the genotype-phenotype relationships become clearer, it will be clinically important to readily subtype the deletion class<sup>56</sup>.

### Clinical manifestations and molecular genetics of PWLS

PWLS share features of the PWS phenotype, however the genetic basis of these rare disorders differs. The implication is that the gene functions disrupted in PWLS are likely to lie in genetic pathways that are important for the development of PWS phenotypes. The main clinical manifestations of PWLS were reported including hypotonia, obesity, short extremities, and delayed development<sup>74</sup>. However, PWLS phenotype is clinically and genetically heterogeneous. Recently, several patients with PWLS (ASD, intellectual disability, and a varying degree of clinical and behavioral features of PWS) were reported in whom four different *de novo* heterozygous truncating mutations in the *MAGEL2* gene were identified which occurred on the paternal allele, suggesting that *MAGEL2* is a novel gene causing complex ASD, and *MAGEL2* loss of function can contribute to several aspects of the PWS phenotype<sup>14</sup>. Rocha, et al.<sup>52</sup> reviewed a total of 117 PWLS patients. Of these patients, 44 had their final genetic diagnosis established. Their most frequent symptoms were obesity (84%), hyperphagia (72.7%), mental disability (54.5%), psychomotor delay (50%), and hypotonia (43.18%). It is important to recognize that signs and symptoms of PWS could also be found in patients who show other types of chromosomal abnormalities. For example, chromosomal abnormalities such as chromosome 14 maternal uniparental disomy<sup>75</sup>, 1p36 monosomy<sup>76</sup>, deletion

of 6q<sup>74,77,78</sup>, 2pter deletion<sup>79</sup>, 10q26 deletion<sup>80</sup>, paracentric inversion (X)(q26q28)<sup>81</sup>, 12q subtelomere deletions<sup>82</sup>, Xq27-qter disomy, deletion of 3p26.3<sup>22</sup>, fragile X<sup>83</sup>, fragile X with 47,XY<sup>84</sup>, duplication of X(q21.1-q21.31)<sup>85</sup>, and a duplication of Xq23-q25 duplication<sup>86</sup> could be associated with the PWLS phenotype. Genes that are outside of the PWS region result in PWLS phenotypes, and are thus implicated in important pathways in PWS pathobiology that might require additional research. For example, mutations in *SIMI*, leptin receptor (*LEPR*), pro-opiomelanocortin (*POMC*), melanocortin 4 receptor (*MCR4*), and more recently, *POU3F2* have all been related to severe obesity which suggests a convergence onto the leptin-melanocortin pathway in association with oxytocin<sup>87,88</sup>. Among them, the *SIMI* gene, mapping to the common 6q16.2deletion region, has been proposed as a candidate for the obesity observed in all these subjects. The 4.1 Mb critical region for PWS includes *SIMI* but also 11 other genes or gene predictions, and the specific role of *SIMI* haploinsufficiency in the development of PWS has not definitively been established<sup>77</sup>. Bonnefond et al.<sup>89</sup> sequenced *SIMI* gene in 44 children with PWLS features, 198 children with severe early-onset obesity, 568 morbidly obese adults, and 383 controls. They identified 4 rare variants (p.I128T, p.Q152E, p.R581G, and p.T714A) in 4 children with PWLS features (including severe obesity) and 4 other rare variants (p.T46R, p.E62K, p.H323Y, and p.D740H) in 7 morbidly obese adults. Three mutations showed strong loss-of-function effects (p.T46R, p.H323Y, and p.T714A) and were associated with high intra-family risk for obesity, while the variants with mild or no effects on *SIMI* activity were not associated with obesity within families. It suggested a firm link between *SIMI* loss of function and severe obesity associated with, or independent of, PWS-related clinical features<sup>53</sup>. Al Ageeli et al.<sup>90</sup> reported case with *SMC15* of paternal origin and PWLS features: motor and intellectual delay, autism, auto-aggressivity, attention deficit and excessive eating, obesity, and facial dysmorphism (round face, deep set eyes, narrow palpebral fissures, epicanthus, and upturned nose with broad nasal bridge). A comparative study investigating the prevalence and severity of obsessive-compulsive symptoms (OCS) in PWS and PWLS showed that PWS patients suffered a higher incidence of OCS and more severe symptoms than their PWLS counterparts<sup>91</sup>.

### Clinical diagnosis and diagnostic testing

Even though consensus clinical diagnostic criteria for PWS were established in 1993, 17% of 90 patients with a molecular PWS diagnosis did not fulfill the consensus clinical diagnostic criteria<sup>92</sup>. Therefore, they suggested new criteria to prompt DNA testing for PWS (cognitive impairment, excessive eating, central obesity, and hypothalamic hypogonadism). DNA methylation analysis is the most efficient way to start the genetic workup if PWS is suspected clinically (Fig. 2). DNA methylation analysis is the only technique that will diagnose PWS in all 3 molecular classes and differentiate PWS from AS in deletion cases, and a methylation analysis consistent with

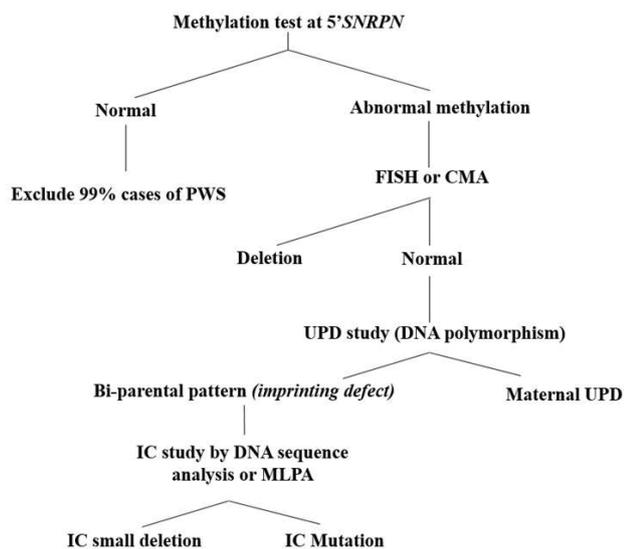
PWS is sufficient for clinical diagnosis<sup>16,20</sup>. The most robust, and now most widely used, assay targets the 5' CpG island of the *SNURF-SNRPN* locus, and it will correctly diagnose PWS in more than 99% of cases<sup>20</sup>. Although, the methylation analysis is the gold standard technique for detecting PWS, it cannot distinguish the molecular class (i.e., deletion, UPD, or ID). Therefore, cytogenetic analysis should also be performed, not only to look for a 15q11-q13 deletion, but to find other chromosomal abnormalities<sup>52</sup>. For genetic counseling purposes, a chromosomal analysis is also recommended in the proband to discern an interstitial *de novo* deletion from a balanced or unbalanced chromosomal rearrangement involving the 15q11.2 region. Traditionally, deletions of 15q11.2-q13 have been diagnosed with Fluorescence in situ hybridization (FISH) with the *SNRPN* probe<sup>15</sup>. With the increasing use of chromosomal microarray (CMA) in clinical genetics, it is possible that arrays may replace FISH analysis for the identification of deletions in PWS and AS<sup>15</sup>. CMA will precisely report not only the deletion size, but also additional chromosomal abnormalities elsewhere in the genome, which is anticipated to become increasingly important for genotype-phenotype correlations in the future<sup>44</sup>. However, CMA will not identify the rare chromosomal rearrangements (translocations and inversions) involving proximal 15 which are detectable by simultaneous karyotype and FISH analysis and are important in recurrence risk determination.

If DNA methylation is positive for PWS (i.e., maternal only imprint), but no deletion is found, the next step is to distinguish between maternal UPD and an ID<sup>15</sup>. This is accomplished by using DNA polymorphism analysis of chromosome 15 loci on the proband's and parents' DNA, which can diagnose

UPD in some cases but not all<sup>93</sup>. If the family polymorphism study reveals that the proband has biparental inheritance of chromosome 15 loci (rather than maternal UPD), then the molecular class is presumed to be an ID<sup>15</sup>. It is then important to determine whether the ID is due to an epimutation (low recurrence risk) or a small deletion in the IC, as in the later situation the recurrence risk can be as high as 50% if the father also has an IC deletion<sup>15</sup>. Approximately 15% of individuals with PWS due to ID have an IC deletion and approximately 50% of these are familial mutations<sup>78</sup>. Testing for IC deletion can be done by sequence analysis at the smallest region of overlap for the PWS IC, which is a region of approximately 4.3 kb<sup>63,78</sup>, or by the recently developed methylation-specific multiplex-ligation probe amplification (MS-MLPA) assay. Some laboratories, particularly in Europe, now begin testing for PWS by using the MS-MLPA analysis (rather than single locus DNA methylation analysis at 5'*SNRPN*), with a detection rate of more than 99%<sup>15</sup>. Compared to traditional DNA methylation, the advantage of MS-MLPA is that the MS-MLPA will investigate 5 distinct differentially methylated sites rather than just one locus and will give information on dosing in the 15q11.2 region<sup>15</sup>. The latest kit has particularly dense probe coverage for dosing and DNA methylation analysis in the PWS "critical region" between the PWS IC and *SNORD116*<sup>15</sup>. In addition, the MS-MLPA technique is much more labor/cost-effective than CMA analysis, although CMA provides more precise information regarding the extent of the deletion<sup>44</sup>. Therefore, MS-MLPA analysis might be considered as the first testing when suspecting AS or PWS as a possible diagnosis, especially since important genotype-phenotype correlations will likely be forthcoming<sup>44</sup>. However, MS-MLPA will also not detect chromosomal rearrangements (inversions and translocations) involving proximal 15, which are detectable by simultaneous karyotype and FISH analysis<sup>15</sup>.

## Genetic counseling

Understanding the specific genetic etiology in individuals with PWS is vital for the appropriate genetic counseling of affected families<sup>15</sup>. Estimation of recurrence risk is dependent on the genetic defect causing PWS. Deletion 15q11.2-q13 is sporadic (recurrence risk <1%) except in the rare cases where a chromosomal balanced rearrangement (translocation or inversion) is present in the father<sup>94</sup>. In these cases, there is a theoretical recurrence risk as high as 25%–50%. In addition, a scenario with a risk of 100% is very unlikely but theoretically possible (i.e., a mother with a 15/15 Robertsonian translocation). Although chromosome rearrangements are the most infrequent genetic cause (<1%), it is important to analyze the karyotype of patients suspected of PWS to identify chromosome 15 rearrangements, plus other chromosomal anomalies because the karyotype and FISH analyses carried out in the affected child could give enough information to suspect whether the deletion comes from a chromosome rearrangement<sup>95,96</sup>. Small supernumerary marker chromosomes have been reported in ~0.3% of mentally retarded patients<sup>97</sup>, and in most cases, the



**Fig. 2.** Diagnostic algorithm in patients suspected of Prader-Willi syndrome (PWS). FISH, fluorescence in situ hybridization; CMA, chromosomal microarray; UPD, uniparental disomy; IC, imprinting center; MLPA, multiplex ligation probe amplification.

small supernumerary marker chromosomes could be derived from chromosome 15, resulting in a UPD<sup>61</sup>). Only in these cases are studies on fathers recommended to offer a thorough genetic counseling<sup>98</sup>. Maternal UPD 15 is typically *de novo* (recurrence <1%) except if a Robertsonian translocation is present in either parent. If the patient chromosomal analysis is normal, it must be expected that the maternal UPD be sporadic<sup>98</sup>. Taking together, there are two important considerations. First, the patient chromosomal analysis is also important to identify the presence of small supernumerary marker that could explain some maternal UPD cases. Second, if a Robertsonian translocation is identified in the patient karyotype and suspected as the origin of a maternal UPD, then it is the mother karyotype that must be performed instead of the father one<sup>97</sup>. Approximately 15% of those with an imprinting defect have a microdeletion in the IC; this can be familial and has a 50% recurrence risk when it is<sup>97</sup>. Therefore, fathers of children with an IC deletion should have DNA methylation and dosing analysis (or sequence analysis) to determine whether they carry the IC deletion<sup>15</sup>. However, the greater proportion of those with an imprinting defect have an epigenetic mutation and the recurrence risk is <1% for this group<sup>98</sup>.

## Conclusions

A wide range of genetic conditions are associated with appetite control, body composition, growth, reproduction, learning disability, psychosis and other behavioral problems. As yet, many outstanding questions remains to be answered about these condition although much is being learnt relating to the important contribution of specific genes, and their coded proteins. The genetic complexity of the PWS chromosomal region, with multiple imprinted genes, alternative splice variants, gene duplications and variant copies, and the mechanisms of imprinting itself, are matched by the wide variety of phenotypes that involve multiple organ systems. Updated information regarding the genetics and phenotypic characterization of individuals with PWS and PWLS is important for all physicians and will be helpful in recognizing patients that may benefit from further investigation and genetic screening and anticipating complications associated with this rare obesity-related disorder.

## Conflict of interest

No potential conflict of interest relevant to this article was reported.

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