Preclinical Testing in Translational Animal Models of Prader-Willi Syndrome: Overview and Gap Analysis

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Prader-Willi syndrome (PWS) is a rare neurodevelopmental disorder causing endocrine, musculoskeletal, and neurological dysfunction. PWS is caused by the inactivation of contiguous genes, complicating the development of targeted therapeutics. Clinical trials are now underway in PWS, with more trials to be implemented in the next few years. PWS-like endophenotypes are recapitulated in gene-targeted mice in which the function of one or more PWS genes is disrupted. These animal models can guide priorities for clinical trials or provide information about efficacy of a compound within the context of the specific disease. We now review the current status of preclinical studies that measure the effect of therapeutics on PWS-like endophenotypes. Seven categories of therapeutics (oxytocin and related compounds, K⁺-ATP channel agonists, melanocortin 4 receptor agonists, incretin mimetics and/or GLP-1 receptor agonists, cannabinoids, ghrelin agents, and Caralluma fimbriata [cactus extract]) have been tested for their effect on endophenotypes in both PWS animal models and clinical trials. Many other therapeutics have been tested in clinical trials, but not preclinical models of PWS or vice versa. Fostering dialogs among investigators performing preclinical validation of animal models and those implementing clinical studies will accelerate the discovery and translation of therapies into clinical practice in PWS.

The Multigene Nature of PWS

The majority of cases of PWS are caused either by a sporadic deletion of a set of genes on the paternally inherited copy of chromosome 15q11-q13 or by maternal uniparental disomy of chromosome 15 (Figure 1). A minority of cases are caused by sporadic or inherited mutations within an embedded regulatory region called the imprinting center (IC). The PWS region contains protein-coding genes and non-coding RNAs and genetic elements that coordinate gene expression and imprinting. Like some other microdeletion disorders, individual genes within the deleted region are implicated in specific endophenotypes, but disruption of several genes is required to elicit the full phenotype. All but one (NPAPI) of the PWS protein-coding genes are conserved in other mammals, while the non-coding elements vary in the extent of their sequence and functional conservation (Figure 1). The genetic complexity of the deleted region...
| Strain       | Treatment          | Treatment Administration | Note | Outcome                  | Group Size (M/F) | Age          | Reference | Title                                                                 |
|-------------|--------------------|--------------------------|------|--------------------------|-----------------|--------------|-----------|-----------------------------------------------------------------------|
| Magel2tm1.1Mus | oxytocin           | i.p. single injection, 2 µg | a    | survival                 | n = 33–51, M and F | 3–5 h        | 5         | A single postnatal injection of oxytocin rescues the lethal feeding behavior in mouse newborns deficient for the imprinted Magel2 gene |
| Magel2tm1.1Mus | oxytocin           | i.p. daily, 7 days, 2 µg | b    | survival, social behavior | n = 12–14, M    | 0–6 d, test adult | 29        | An early postnatal oxytocin treatment prevents social and learning deficits in adult mice deficient for Magel2, a gene involved in PWS and autism |
| Magel2tm1.1Mus | oxytocin           | i.p. single injection, 2 µg | c    | social recognition deficits | n = 12–14, M    | adult        | 29        | An early postnatal oxytocin treatment prevents social and learning deficits in adult mice deficient for Magel2, a gene involved in PWS and autism |
| Magel2tm1Stw  | MT-II              | i.p. single injection, 2.5 mg/kg | d    | 24 h food intake         | n = 6, M        | 3–4 months   | 6         | Magel2 is required for leptin-mediated depolarization of POMC neurons in the hypothalamic arcuate nucleus in mice |
| Magel2tm1Stw  | setmelanotide      | i.p. single injection (0.04, 0.1, 0.2 or 1 mg/kg) | e    | food intake, EE, RER     | n = 6, M        | 2–3 months   | 34        | Magel2-null mice are hyper-responsive to setmelanotide, a melanocortin 4 receptor agonist |
| Magel2tm1Stw  | sleeve gastrectomy | surgery                  | f    | weight, food intake, fat/lean mass, fasting glucose, glucose tolerance, counter-regulation | n = 7–12, M    | 5–7 months, HFD | 35        | Sleeve gastrectomy leads to weight loss in the Magel2 knockout mouse |
| Magel2tm1Stw  | JD5037 or SLV319   | i.p. daily 28 d, 3 mg/kg | g    | weight, food intake, body comp. activity, metabolism | n = 5–10, M and F | 3–4 months, HFD or STD | 36        | Targeting the endocannabinoid and CB1 receptor system for treating obesity in PWS |
| Magel2tm1Stw  | OEA                | i.p. single injection, 10 mg/kg | h    | 24 h food intake         | n = 14–15, M    | adult        | 39        | Dysfunctional oleoylethanolamide signaling in a mouse model of PWS |
| Magel2tm1Stw  | diazoxide          | ground into food, 150 mg/kg/day, 6 weeks | i    | weight, body composition, activity, fasting glucose | n = 6, M and F  | 5–7 months, HFD | 38        | Chronic diazoxide treatment decreases fat mass and improves endurance capacity in an obese mouse model of PWS |
| Magel2tm1Stw  | oleoyl a-methyl serine (HU-671) | once daily, 0.5 mg/kg, 6 weeks | j    | structural analysis of the trabecular and cortical bones | n = 4–11, F    | 6–12 weeks   | 37        | Magel2 modulates bone remodeling and mass in Prader-Willi syndrome by affecting oleoyl serine levels and activity |
| Ndn tm1.1Mus  | fluoxetine         | 10 mg/kg/day from P5–P15 | k    | plethysmography (% with apnea, apnea/h, apnea duration) | n = 8, M and F  | 5–15 d, measure 0, 15, 45 days later | 71        | Necdin shapes serotonergic development and SERT activity modulating breathing in a mouse model for PWS |
| Snord116tm1.1Usa | [D-Lys3]-GHRP6     | i.p. daily, 12 µmol/kg, 6 days | l    | food intake              | n = 7–9, M      | 6–12 months   | 113       | Abnormal response to the anorexian effect of GHS-R inhibitors and exenatide in male Snord116 deletion mouse model for PWS |
| Snord116tm1.1Usa | SPA                | i.p. daily, 4.5 µmol/kg, 6 days | m    | food intake              | n = 8, M        | 6–12 months   | 113       | Abnormal response to the anorexian effect of GHS-R inhibitors and exenatide in male Snord116 deletion mouse model for PWS |

(Continued on next page)
| Strain                | Treatment                  | Treatment Administration | Note | Outcome                  | Group Size (M/F) | Age          | Reference | Title                                                                                           |
|----------------------|----------------------------|----------------------------|------|--------------------------|-----------------|--------------|-----------|------------------------------------------------------------------------------------------------|
| Snord116<sup>tm1.1Uta</sup> | YIL-781                   | i.p. daily, 134 µmol/kg, 6 days | a    | food intake              | n = 9–10, M     | 6–12 months | 113       | Abnormal response to the anorexic effect of GHS-R inhibitors and exenatide in male Snord116 deletion mouse model for PWS |
| Snord116<sup>tm1.1Uta</sup> | exenatide                  | i.p. twice daily, 24 µg/kg, 17 days | a    | food intake              | n = 7–9, M      | 6–12 months | 113       | Abnormal response to the anorexic effect of GHS-R inhibitors and exenatide in male Snord116 deletion mouse model for PWS |
| Snord116<sup>tm1.1Uta</sup> | GHSR agonist HM01         | s.c. daily, 30 µg/g, 14 days | q    | body weight, length, mortality | n = 9–17, M and F | 1–14 days | 114       | Ghrelin receptor agonist rescues excess neonatal mortality in a Prader-Willi syndrome mouse model |
| Snord116<sup>tm1.1Uta</sup> | Carraluma fimbriata extract | orally in gel, 33 or 100 mg/kg/day, 10 weeks | q    | food intake after stimulation | n = 6, M and F | 4–15 weeks | 165       | Carraluma fimbriata extract activity involves the 5-HT2c receptor in PWS Snord116 deletion mouse model |
| Snord116<sup>tm1.1Uta</sup> | thermoneutral (30°C)      | 16 weeks                   | t    | body weight, body comp, length, BMD, energy intake | n = 9–14, M from 4 to 20 weeks | 106       | Ambient temperature modulates the effects of the PWS candidate gene Snord116 on energy homeostasis |
| Snrpn<sup>Del(7Ube3a-Snrpn)1Alb</sup> | thermoneutral (30°C)    | 9 weeks                   | t    | food intake, fat mass, weight gain | n = 5–6, M and F | 6–15 months | 118       | Increased alternate splicing of Htr2c in a mouse model for Prader-Willi syndrome leads disruption of 5HT2c receptor mediated appetite |
| Snrpn<sup>Del(7Ube3a-Snrpn)1Alb</sup> | WAY-161503                | s.c. single injection, 3 mg/kg or 10 mg/kg | t    | postfast refeeding       | n = 12–14, M and F | adult | 122       | Targeting the histone methyltransferase G9a activates imprinted genes and improves survival of a mouse model of PWS |
| Del(7Ube3a-Snrpn)1Alb | UNCO0642                  | i.p. daily P7–P12, 2.5 mg/kg | a    | % survival, body weight  | n = 6–27, M and F | 7–90 days   | 123       | Restoration of bone density and bone mass, decreased bone resorption, and increased bone formation |

1, i.p., intraperitoneal; s.c., subcutaneous; EE, energy expenditure; RER, respiratory exchange ratio; HFD, high-fat diet; SD, standard diet; BMD, bone mineral density; P, postnatal day.

*Rescued the death rate of Magel2 mice with a single injection of oxytocin 3–5 h after birth.

*Increased BMD and BMC, high energy expenditure and low physical activity, but not low body weight, in Snord116 mice.

*Reduced food intake only on day 1 in WT and Snord116 mice.

*Reduced food consumption in WT but not PWS-ICdel mice compared to vehicle.

*Reduced food intake only on day 1 in WT and Snord116 mice.

*Food intakes for WT and Snord116 mice were reduced to ~84%.

*GHBP, growth hormone secretagogue receptor. Reduced body weight and length in male Snord116 pups, reduced mortality in Snord116 pups.

*Similar weight loss in both Magel2 and WT mice by specifically causing loss of fat but not lean mass. Lowered fasting glucose and improved glucose tolerance in both WT and Magel2 mice.

*Reduced obesity, reduced hyperphagia, increased total energy expenditure and voluntary activity, food intake and carbohydrate intake, and improved metabolic outcomes in obese Magel2 mice.

*Reduced food intake in Magel2 mice, compared to vehicle. This effect was still evident 24 h after injection. Lesser extent of reduced food intake for the first 2 h of refueling in control mice. This effect was no longer present by 4 h.

*Reduced food intake, increased energy expenditure, and increased activity in WT and Magel2 mice compared to vehicle. Magel2 mice responded at lower dosages of setmelanotide.

*Reduced energy intake and increased physical activity in WT and Magel2 mice. Both WT and Magel2 mice showed increased locomotor activity.

*Increased alternate splicing of Htr2c in a mouse model for Prader-Willi syndrome leads to hyperphagia and obesity.

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*Reduced food intake, increased energy expenditure, and increased activity in WT and Magel2 mice compared to vehicle. Magel2 mice responded at lower dosages of setmelanotide.

*Increased alternate splicing of Htr2c in a mouse model for Prader-Willi syndrome leads to hyperphagia and obesity.
Complicates the development of animal models of PWS. It is thus useful to describe the individual genes whose expression is absent in PWS, evaluate the possible contribution of each gene to endophenotypes in PWS, and describe preclinical studies in mice carrying mutations targeting these genes.

MAGEL2

MAGEL2 (melanoma antigen gene L2) encodes a member of the melanoma antigen (MAGE) protein family. MAGE proteins are defined by a conserved 171-amino-acid domain (MAGE homology domain [MHD]) that interacts with other proteins. MAGEL2 is expressed primarily in the hypothalamus, the part of the brain that controls appetite, endocrine function, and homeostatic functions. MAGEL2 is also expressed in the peripheral nervous system, developing muscle, cartilage, and bone. MAGEL2 is predicted to encode a 1249-amino-acid protein, although endogenous MAGEL2 protein has not yet been detected in tissues. One of the cellular roles of MAGEL2 is as an adaptor protein for E3 ubiquitin ligases and deubiquitinases.

Since 2012, additional phenotypes have been uncovered in Magel2 mutant mice. Magel2<sup>tm1Stw</sup> mutant mice spent more time in the open arm of an elevated plus maze compared to wild-type (WT) littermates, suggesting reduced anxiety, and had a lack of preference for the anorexigenic effects of injected leptin hormone. More recently, adult Magel2<sup>tm1Stw</sup> mutant mice are insensitive to the anorexigenic effects of injected leptin hormone. Moreover, their hypothalamic POMC neurons do not depolarize in response to leptin. Magel2<sup>tm1.1Mus</sup> mutant mice demonstrated sucking defects causing slow growth or lethality in pups.

**Mouse Models of Magel2 Deficiency**

Two mouse models of Magel2 deficiency have been developed. The Magel2<sup>tm1.1Mus</sup> mouse line carries a lacZ insertion that replaces the C-terminal domain of the Magel2 open reading frame, including the MHD (JAX stock: 009062). The Magel2<sup>tm1.1Mus</sup> line carries a deletion of the Magel2 promoter and most of the open reading frame. Magel2<sup>tm1Stw</sup> mutant mice have physiological abnormalities including disintegration of circadian rhythm in constant lighting conditions, infertility, reduced strength and locomotor activity, increased fat mass with decreased muscle mass, abnormal hypothalamic-pituitary-adrenal function, reduced counter-regulatory response to hypoglycemia, reduced levels of Igf1 and thyroid hormones, abnormal brain structure, abnormal levels of dopamine and serotonin pathway compounds in brain tissues, and abnormal behavior. Consistent with their excess fat mass, adult Magel2<sup>tm1Stw</sup> mutant mice are insensitive to the anorexigenic effects of injected leptin hormone. Moreover, their hypothalamic POMC neurons do not depolarize in response to leptin. Magel2<sup>tm1.1Mus</sup> mutant mice demonstrated sucking defects causing slow growth or lethality in pups.

**Figure 1. Genes Implicated in Prader-Willi Syndrome**

(A) Paternally expressed, imprinted genes located within the PWS deletion region are indicated on a genomic map of human chromosome 15q11-13. Protein coding genes and non-coding RNAs are indicated as circles and vertical lines, respectively. Common breakpoints (BP; X) are found in cases of PWS by deletion of 15q11-q13. (B) The mouse chromosome 7C region has conserved synteny with the human PWS region with a few exceptions: mice do not have a homolog of human NPAP1, and Frat3 occurs exclusively in rodents. The six PWS mouse models in which intervention studies have been performed are indicated below the genomic map, with the approximated location and size of the gene-targeted deletions indicated by triangles. cen, centromere; IC, imprinting center (black box); tel, telomere.
function of hypothalamic anorexigenic circuits. As well, Mage2tm1Stw mutant mice demonstrate dopamine pathway imbalances and histological and functional muscle impairment, including a progressive reduction in limb strength and endurance with age. Tissues from Mage2tm1Stw mutant mice contain an increased number of p62 aggregates in skeletal muscle and reduced proportion of p62-positive POMC-expressing neurons in the arcuate nucleus. This suggests abnormal autophagy is occurring in skeletal muscle and in the brain. Abnormal levels of proteins important for recycling of the leptin receptor were found in brain tissues from Mage2tm1Stw mutant mice, consistent with a role for MAGEL2 in membrane protein recycling. There is compelling evidence that inactivation of MAGEL2 is the major cause of endocrine, musculoskeletal, and neurological dysfunction in PWS. The high expression of MAGEL2 in the parts of the nervous system that are dysfunctional in PWS, the importance of MAGEL2 protein function in the neurological, endocrine and musculoskeletal systems, and the striking similarities between phenotypes in Mage2 mutant mice and individuals in PWS all support the hypothesis that loss of MAGEL2 makes a major contribution to PWS.

Interventional Studies in Mage2 Mutant Mice
Nine studies have examined the effect of therapeutic interventions in Mage2 mutant mice (Table 1). A postnatal injection of oxytocin improved survival of Mage2tm1MUs mutant mice, which otherwise have an early postnatal mortality rate of about 50%. In a follow-up study, early postnatal treatment of Mage2tm1MUs mutant mice with oxytocin prevented the social and learning deficits that adult Mage2tm1MUs mutant mice would otherwise display. Administration of MT-II, a melanocortin 4 receptor agonist, decreased food intake in Mage2tm1Stw mutant mice to a greater extent than in WT controls. In a follow-up study, Mage2tm1Stw mutant mice were hyper-responsive to setmelanotide, a newer melanocortin 4 receptor agonist, with a greater reduction in food intake after a single dose compared to WT. Mage2tm1Stw mutant mice responded to bariatric surgery (sleeve gastrectomy) with loss of fat mass and improved metabolic parameters. Administration of JDS037, which targets the endocannabinoid and CB1 receptor system, reduced fat mass in Mage2tm1Stw mutant mice. Mage2tm1Stw mutant mice suffer from a low-bone-mass phenotype, but KAL671 (oleoyl α-methyl serine), an endocannabinoid-like compound, prevented trabecular bone loss in these mice. Mage2tm1Stw mutant mice have high fat mass and reduced endurance, phenotypes that were ameliorated by chronic diazoxide treatment. Mage2tm1Stw mice have dysfunctional oleoyl-thanolamide signaling, and intraperitoneal administration of oleoyl-thanolamide significantly reduced food intake on fasting and refeeding. Overall, these results suggest that Mage2 mutant mice recapitulate many of the phenotypes of both Schaaf-Yang syndrome and PWS and are an excellent model for preclinical testing of therapeutics destined for clinical trials in these disorders.

NDN
NDN (neurally differentiated embryonal carcinoma-derived protein) encodes necdin, which is another member of the MAGE protein family containing a C-terminal MHD. Necdin was first described as a protein that is highly expressed in neurally differentiated embryonal carcinoma cells. Necdin interacts with a variety of proteins and plays roles in transcription, cell cycle entry, and cell-surface receptor signaling. In one study, a de novo NDN variant (p.Ala280Pro) was found in a patient with Smith-Magenis-like syndrome. It is unclear whether this variant is pathogenic on its own or in combination with a de novo variant in MAPK8IP3 identified in the same individual.

Mouse Models of Ndn Deficiency
Four lines of mice carrying loss-of-function mutations in Ndn have been described: Ndn^tm1.1MUs, Ndn^tm1Alb, Ndn^tm1Ky (repository RBRC02316), and Ndn^tm2Stw (JAX stock: 0090899). All four lines carry deletions within the coding region, and the promoter is also deleted in one of the lines (Ndn^tm1.1MUs). Prior to 2012, phenotypes described in Ndn mutant mice that recapitulate findings in PWS include severe neonatal respiratory compromise, behavioral abnormalities, abnormalities of central, autonomic, and peripheral nervous system development and function, enlarged brain ventricles, and abnormal development of limb musculature. Necdin regulates preadipocyte proliferation in developing adipose tissues, and Ndn^tm1Ky become obese with adipocyte hyperplasia when fed a high-fat diet. Embryonic fibroblasts, cortical neurons, and muscle progenitor cells from Ndn^tm2Stw mutant mice, as well as human PWS fibroblasts, displayed impaired myosin activation and polarization. Interestingly, necdin is important for normal hematopoiesis, although hematopoietic defects are not described in PWS. A last line of mice (tgMlcNec) overexpressed necdin only in skeletal myoblasts and skeletal muscle and were used to show that necdin expression is important for a protective response of the muscle against tumor-induced wasting, inhibition of myogenic differentiation, and fiber regeneration in mice with cachexia. Necdin also modulates osteogenic cell differentiation by regulating two other genes, Dlx5 and Maged1.

In the last five years, studies of mice carrying Ndn mutations have confirmed that necdin is an essential protein for physiological processes relevant to PWS phenotypes. Ndn^tm1.1MUs mutant mice have severe neonatal respiratory deficiency that is central in origin and is caused by serotonergic dysfunction. Hypotonia, necdin enhances myoblast survival by facilitating the degradation of the mediator of apoptosis CCAR1/CARP1, in a study that used both tgMlcNec mice and Ndn^tm2Stw mice. Necdin regulates the quiescence and response to genotoxic stress of hematopoietic stem and progenitor cells, as demonstrated using Ndn^tm2Stw mutant mice. Necdin also protects neurons against mitochondrial insults by promoting mitochondrial biogenesis, in a study that used the Ndn^tm1Ky mice. Necdin controls epidermal growth factor receptor signaling linked to astrocyte differentiation, interacts with the RING finger protein Bmi1 to control the proliferation of neural precursor cells in the neocortex, and modulates the thyroid axis. Along with MAGE2, necdin facilitates leptin receptor recycling, as shown in cultured cells and in mouse brain tissue. Lastly, functional interactions between necdin and other proteins have been uncovered,
including interactions with the alpha subunit of the guanine nucleotide-binding protein Go52 and the ciliary protein cystin.83 Thus, necdin is implicated in many physiological processes that are relevant to the complex clinical features in PWS.

**Interventional Studies in NDN Mice**

Only one study has described an interventional trial in Ndn mutant mice. Serotonin deficiency in newborn mice causes neurological, respiratory, and behavioral abnormalities.84 Fluoxetine (Prozac) is an antidepressant and selective serotonin reuptake inhibitor that increases extracellular serotonin levels (Table 1). Many Ndnmutant mice present with apnea at birth and have more apneas per hour and a higher accumulated apnea duration compared to WT. Respiratory deficits in mutant pups were suppressed by transient treatment with fluoxetine.71 The researchers suggest that respiratory complications in PWS infants could respond to therapeutics that target the serotonin system.11

**SNHG14, SNORD116, and IPW**

SNHG14 (small nucleolar RNA host gene 14) is a long non-coding RNA (lncRNA) initiated at the SNRPN gene. SNHG14 is also known as LNCAT or U-UBE3A-ATS.85–90 A cluster of snoRNAs is generated from the SNHG14 introns, with the best studied of these being the SNORD116 (small nucleolar RNA, C/D box 116; previously known as HBII-85) cluster.91 SnoRNAs are nuclear RNAs present in ribonucleoprotein complexes (snoRNPs) that function to modify other RNAs or participate in ribosomal RNA maturation. IPW (imprinted in Prader-Willi)92,93 is a stable non-coding RNA generated from three exons of the SNHG14 lncRNA. Several individuals with atypical PWS carrying chromosomal deletions, as small as 118 kb, within SNHG14 have been described.94 In the case with the smallest deletion, expression of SNORD116 (and likely also IPW) was abolished, while expression of SNRPN was intact.74 The deletions within SNHG14/SNORD116 cluster could modify the expression of MAGEL2 through long-range chromatin interactions.95 This would explain how micro-deletions that include the SNORD116 cluster produce phenotypes that overlap with Schaaf-Yang syndrome caused by MAGEL2 mutations.

**Mouse Models of Snord116 and Ipw Deficiency**

Two lines of mice were developed to assess Snord116 function: B6(Cg)-Snord116tm1Jbro and Del(7Ipw-Snord116)tm1Jbro.97 Other transcripts, such as the murine equivalents of SNHG14 and IPW,75,76 are also disrupted in both of these mutant mouse lines. Abnormal phenotypes affecting the neuronal, endocrine pancreas, bone mass, cognitive, and behavioral systems were discovered in Snord116mutant mice.99–102 While these mice have impaired growth, they do not display hyperphagia or obesity, nor do they have defects in the hypothalamic leptin or melanocortin systems.103 A deficiency in prohormone convertase (PC1, encoded by Pcsk1) and impaired prohormone processing was identified in Snord116mutant mice in one study.104 Another study of the same mice revealed no differences in hypothalamic Pcsk1 expression in either fed or fasted states.105,106 Interestingly, the hypothalamus-specific reintroduction of Snord116 into Snord116mutant mouse increased energy expenditure.105,106

Adult-onset deletion of Snord116 in conditional (floxed) Snord116mutant mice resulted in reduced feeding and increased fat mass in one study.107 In another study with the same mice, virally induced cre-mediated deletion of Snord116 increased food intake and body weight in a subset of adult treated mice.108 These seemingly contradictory results have yet to be resolved. In Snord116mutant mice, the lncRNA was shown to modulate diurnal gene expression, DNA methylation, and energy expenditure.108,110 Working-for-food behaviors and sleep patterns were abnormal in Del(7Ipw-Snord116)tm1Jbro mutant mice.111,112 More studies are needed to resolve the possible role of Snord116 in the hypothalamic and circadian regulation of energy balance.

**Interventional Studies in Snord116 Mutant Mice**

Four studies have described therapeutic interventions in Snord116 mutant mice (Table 1). Snord116mutant mice had an abnormal response to the anorexie effect of growth hormone secretagogue receptor inhibitors and to exenatide, a glucagon-like peptide-1 receptor agonist.113 In the same mice, a growth hormone secretagogue (ghrelin) receptor agonist (HM01) rescued excess postnatal, preweaning mortality.114 These data support exploration of the therapeutic potential of ghrelin receptor agonist administration in the failure to thrive period of PWS. An extract of cactus (Caralluma fimbriata, CFE) was fed to a cohort of Snord116mutant mice, and the effect on 4-h food intake was measured after 9 weeks of treatment. CFE appeared to decrease food intake in Snord116mutant mice, although differences between mutant and WT untreated mice in their responses to appetite stimulants complicate the interpretation of these results. Another study showed that housing Snord116mutant mice at a thermoneutral temperature (30°C) instead of ambient temperature commonly maintained in animal facilities, normalized many phenotypes in these mice, including low bone mineral density, length, food intake, and energy expenditure. This last study demonstrates that the low body mass and low body fat of Snord116 mutant mice causes cold stress in these animals. Phenotypes in Snord116 mutant mice raised at ambient temperature need to be interpreted with caution, as they may reflect cold adaptation rather than being intrinsic to the genetic defect in these animals.115

**SNRPN and the IC**

SNRPN (small nuclear ribonucleotide polypeptide N) encodes a small nuclear ribonucleotide protein that functions in pre-mRNA processing. The SNRPN promoter and coding exons are shared with the SNHG14 non-coding RNA described above. A complex set of regulatory elements collectively referred to as the IC lies upstream of the SNRPN coding exons. There is no evidence that loss of the SNRPN protein itself contributes to phenotypes in PWS. Chromosomal deletions that affect the SNRPN upstream exons and include the IC cause PWS by impairing the allele-specific expression of genes normally subject to imprinting control.

**Mouse Models of SNRPN and IC Deficiency**

Mice were generated that carry a targeted deletion of 35 kb including 16 kb upstream of Snrpn and exons 1–6.116 When this
deletion is paternally derived, the “PWS-ICdel” mice have severe postnatal growth failure and lethality unless maintained on an outbred strain background (JAX stock: 012443, B6.129-Snrpn<sup>tm2Cbr/J</sup>). Surviving PWS-ICdel have reduced locomotor activity and cognitive deficits. They have low fat mass for their body weight and increased thermogenesis, and some of the energy imbalance was rescued by housing the mice in thermoneutral conditions. After an overnight fast, the PWS-ICdel mice consumed more food than WT in the first 30 min of refeeding, perhaps because their small size increases their energy needs at ambient temperature, or because of an underlying mitochondrial dysfunction. Another group generated a line of mice (Del(7Ube3a-Snrpn)<sup>1Ab</sup>) carrying a targeted deletion from Snrpn to Ube3a, but not including the IC. Similar to the Snord116 mice, Del(7Ube3a-Snrpn)<sup>1Ab</sup> mutant mice have severe growth retardation, hypotonia, and high rates of lethality before weaning. The surviving mice were fertile and did not become obese.

**Interventional Studies in PWS-ICdel Mice and pΔS-U Mice**

PWS-ICdel adult mice were housed under thermoneutral conditions (i.e., 30°C rather than room temperature, 20°C–22°C), which abolished the excess food consumption observed in these mice at room temperature. Like the Snord116 mice, phenotypes in PWS-ICdel mutant mice raised at ambient temperature need to be interpreted with caution, as seemingly abnormal feeding behavior may in fact reflect a normal adaptation to cold temperatures. The anorectic effect of a 5-HT₂C-specific agonist, WAY-161503, was investigated in PWS-ICdel mutant mice. WT mice reduced their food intake on treatment with WAY-161503, but no difference in food consumption was observed in the PWS-ICdel mutant mice. This suggests that one or more genes that are inactivated in PWS-ICdel mutant mice are required for normal serotonin receptor 2C function. UNC0642 is a selective inhibitor of euchromatic histone lysine N-methyltransferase-2 (EHMT2, also known as G9a). UNC0642 activated the maternal copy of PWS region genes, including the snorcnucleus cluster Snord116, and improved survival of Del(7Ube3a-Snrpn)<sup>1Ab</sup> mice. The proposed mechanism for this reactivation is a selective reduction of the dimethylation of histone H3 lysine 9 (H3K9me2) at the PWS IC by UNC0642, without changes in DNA methylation.

**Other Genes: MKRN3, NPAP1, and Non-imprinted Genes**

MKRN3 (Makorin ring finger protein 3) is widely expressed throughout the body. MKRN3 encodes a protein that contains a RING zinc finger motif and several other zinc finger motifs and that may function as a E3 ubiquitin ligase. Inactivating mutations in MKRN3 cause familial precocious puberty. There is no mouse model for MKRN3 deficiency published to date. NPAP1 (nuclear pore-associated protein 1) encodes a protein that is closely related to the transmembrane nucleopore gene POM121. NPAP1 has bi-allelic expression in testis but is paternally expressed in fetal brain. NPAP1 is a primate-specific gene, so there are no mouse models for NPAP1. OCA2 is the human homolog of the mouse <i>p</i> (pink-eyed dilution) gene and encodes an integral membrane protein involved in pigmentation. Mutations in OCA2 cause type 2 oculocutaneous albinism. Although OCA2 is outside the imprinted region, OCA2 becomes hemizygous in children carrying a PWS deletion, and this can have deleterious effects. For example, children with the deletion form of PWS who carry a deleterious variant on the remaining OCA2 maternal allele can have phenotypes that range from hypopigmentation to a more severe condition, oculocutaneous albinism with loss of vision. Likewise, some individuals with PWS have larger deletions (type 1 deletions) that include non-imprinted genes (TUBGCP5, NIPA1, NIPA2, and CYFIP1) proximal to the imprinted gene cluster. Haploinsufficiency for these genes may contribute to more severe behavioral phenotypes observed in individuals with type I deletions compared to those with type II deletions who carry two copies of these genes.

**Comparison between Interventional Studies in Humans with PWS versus Animal Models of PWS**

Many mechanisms of action exist for compounds in development for treatment of PWS symptoms. Preclinical studies can provide invaluable information about how animals respond, in vivo, to these potentially effective therapeutics. Conversely, animal testing of compounds already in therapeutic use or clinical trials may accelerate their adaptation into clinical practice or yield insight into the mechanism of action in PWS. Above, we have described 18 compounds or procedures that have been tested in six mouse models of PWS. At least seven of these classes of compounds have also been tested in double-blind placebo-controlled trials in PWS (Table 2).

Clinical trials in PWS were recently reviewed. The largest number of clinical trials in PWS have tested oxytocin or an oxytocin analog (carbetocin, intranasal FE992097), finding positive effects on both hyperphagia and behavior. A trial of belorabiln, a methionine aminopeptidase 2 (MetAP2) inhibitor that promotes loss of fat mass, was terminated in 2016 after adverse events involving excess blood clot formation, while other MetAP2 compounds are in development. An open label 6-month trial of exendin (Byetta, a GLP-1 receptor agonist) in 10 overweight or obese subjects with PWS was recently completed, demonstrating that treatment reduced appetite without any effect on weight loss in the short term. Trials of a controlled release formulation of diazoxide, a K⁺-ATP channel agonist (https://soleno.life) and of AZP-531 (livoletide), an unacylated ghrelin analog (https://www.millendo.com) have only been reported through press releases. Other registered interventional clinical trials have not yet yielded outcomes (reviewed in Miller et al.). These include GLWL-01 for treatment of hyperphagia (NCT03274856), cannabidiol oral solution for treatment of hyperphagia (NCT03274856), co-administration of tesofenpine and metoprolol for reduction of body weight (tesomet, NCT03194495), RM-493 or setmelanotide, and a melanocortin 4 receptor (MC4R) agonist for weight loss and hyperphagia-related behavior (NCT02311673). Many more studies have described the use of other agents in small numbers of individuals with PWS or in open (non-blinded) trials.
| Type of Drug               | Compound                                | Mouse Model of PWS: Reference | Clinical Trials in PWS | Company/Trial Center | Outcomes                                      | NCT or EudraCT                  |
|---------------------------|-----------------------------------------|--------------------------------|------------------------|----------------------|-----------------------------------------------|---------------------------------|
| Neuropeptide hormones     | oxytocin (examples only)                | Magel2: 5                      | phase II               | many sites           | infant suckling; food intake; hyperphagia; behavior; social behavior | NCT03197662                    |
|                           | FDA approved                            | Magel2: 29                     | phase III, 133, 135, 136, 149 |                      |                                | NCT02205034                    |
|                           | carbetocin/FE 992097                    | EU approved                    | phase II               | Ferring              | hyperphagia; behavior                        | NCT02013258                    |
|                           |                                         |                                | not trialed            | Levo Therapeutics    |                                | EudraCT: 2010-022370-14        |
|                           |                                         |                                |                        |                      |                                | EudraCT: 2017-003423-30        |
| K^+ ATP channel agonist   | diazoxide                               | Magel2: 36                     | not trialed            | not trialed          | hyperphagia; behavior                        | NCT02034071                    |
|                           | FDA approved                            |                                |                        |                      |                                | NCT01968187                    |
|                           | DCCR/extended release                    |                                |                        |                      |                                | NCT02895618                    |
|                           | diazoxide choline investigational       |                                |                        |                      |                                | NCT03440814                    |
| Melanocortin 4 receptor agonists | exenatide (Byetta)                  | Magel2: 6                      | phase II               | Rhythm               | weight loss; hyperphagia-related behavior    | NCT02311673                    |
|                           | FDA approved                            |                                |                        |                      |                                | NCT01444898                    |
|                           | RM-493 or setmelanotide                | Magel2: 24                     | phase II               | Children’s Hospital Los Angeles Aintree University Hospital | weight; metabolism             | EudraCT: 2010-023179-25        |
|                           | investigational                         |                                |                        |                      |                                | NCT01444898                    |
|                           | exenatide extended release              | Snord16: 113                   | exploratory, open label phase II |                      | gastric emptying                   | ACTRN12616000710426            |
|                           | (Bydureon)                              |                                |                        |                      |                                |                                 |
|                           | liraglutide (Saxenda)                   | not trialed                    | phase III              | Garvan Foundation, Australia | obesity                         |                                 |
| GLP-1 receptor agonists   | oleoyl ε-methyl serine (HU-671)        | Magel2: 35                     | phase III              | Novo Nordisk         | BMI; hyperphagia; metabolism                | NCT02527200                    |
|                           | investigational                         |                                |                        |                      |                                | EudraCT: 2014-004415-37        |
|                           | JD5037 or SLV-319 (ibipinabant)         | Magel2: 36                     | investigational        |                      |                                |                                 |
|                           | research only                           |                                |                        |                      |                                |                                 |
|                           | OEA (oleoylethanolamide)                | Magel2: 36                     | phase II               | Insys Therapeutics   | hyperphagia, weight                     | NCT02844933                    |
|                           | supplement                              |                                |                        |                      |                                | NCT03458416                    |
|                           | cannabinoid (CBD) oral solution         | Under review by FDA            | phase II               | Cornell Medical College Karolinska University Hospital | obesity                         | NCT010063109                    |
|                           | withdrawn                               |                                |                        |                      |                                | EudraCT: 2007-006305-25        |
|                           | ghrelin receptor agonist HM01           | Snord16: 114                   | phase IIa              | Alize                | blood glucose levels; weight hyperphagia     | EudraCT: 2014-001670-34        |
|                           |                                          |                                |                        |                      | hyperphagia                           |                                 |
|                           | livoletide (AZP-531)                    | phase II                       | phase II               | Miledel Therapeutics  | hyperphagia                           | NCT03790865                    |
|                           | GLWL 01                                 |                                |                        | GLWL Research        | hyperphagia, behavior                   | NCT03274856                    |
| Natural supplement        | cactus extract from Caralluma fimbriata | Snord16: 165                   | phase I                | Victoria University, Australia | hyperphagia; behavior                | ACTRN12611000334909            |
|                           |                                          |                                |                        |                      |                                |                                 |
Improving Translation to Clinical Trials and Reducing Attrition between Preclinical Trials and Clinical Trials

Extrapolating from the last five years of advances in preclinical and clinical trials, we predict that the next decade will see an intensification of efforts to make PWS a treatable condition. Many agents tested in preclinical models of PWS have not been examined in clinical trials, and conversely clinical trials are being pursued for agents that have not been investigated in preclinical models. At least three important lessons have been learned from the last five years of preclinical studies in PWS. The first lesson echoes the words of Dr. Joseph Garner, who argues that "if we want animal models to translate to human outcomes, then we need to start performing animal experiments as if they were human trials." Important recommendations have been developed to address issues of translatability of preclinical studies to clinical trials (e.g., ARRIVE guidelines). However, such recommendations have not yet been universally adopted in the PWS research community. For example, publications should use the proper nomenclature for the animal strain being used, including the stock name, strain background and backcross information, and references to the original descriptions of the model. Authors should refrain from using terms like "PWS mice," and ensure that at the very least, the publication abstract and methods section contain the proper strain name. Justification of cohort sizes using power calculations, inclusion of both sexes of mice, proper blinding to genotype, and statistical analyses are all important factors in preclinical studies. Where possible, preclinical studies should use doses

### Table 3. Compounds Tested in Humans with PWS but Not in Preclinical Models of PWS

| Type of Drug                  | Compound/Procedure                | Clinical Trials in PWS | Company/Trial Center           | Outcomes                                                  | Clinical Trial Registration |
|------------------------------|-----------------------------------|------------------------|--------------------------------|-----------------------------------------------------------|----------------------------|
| Growth hormone               | growth hormone                    | prescribed as needed   | Karolinska University Hospital, Novo Nordisk | body composition; linear growth; bone mineral density; cognitive and adaptive function | NCT0372125 NCT00705172     |
| Stimulant                    | modafinil                         | case series, open label | Hôpital Purpan, Toulouse, France | sleepiness                                                | N/A                        |
| Synthetic somatostatin      | octreotide                        | phase II               | British Columbia's Children's Hospital | BMI; appetite; behavior; ghrelin concentration            | NCT0175305                 |
| Anticonvulsant               | topiramate                         | phase II phase III     | University of Florida-Brain Institute Hopitaux de Paris | self-injurious behavior eating disorders; self-mutilation; irritability and impulsivity; metabolic status | NCT0065923 NCT02810483     |
| Aromatase Inhibitor          | anastrozole                        | phase II               | Hôpital Armand Trousseau, Paris | bone maturation related to pathological adiparche         | NCT01520467                |
| Serotonin-noradrenaline-      | tesomet (tesofensine and metoprolol) | phase II               | Saniona | body weight | NCT03149445 EudraCT:2016-003694-18                      |
| dopamine reuptake inhibitor/beta-blocker | | | | | |
| Ghrelin pathway              | GLWL-01 investigational           | phase II               | GLWL Research | hyperphagia, HQ-CT score | NCT03274856 | NCT01818921 NCT02179151 EudraCT:2015-005665-33 |
| Methionine aminopeptidase 2 inhibitor | beloranib | phase II phase III | Zafgen | HQ-CT; weight; fat and lean; QOL | NCT03277157 | N/A |
| Probiotic                    | Bifidobacterium lactis B94 (probiotic) supplement | phase II | University of Florida, Gainesville | stool frequency | NCT01863017 NCT0324906 NCT02758262 |
| Brain stimulation            | vagal nerve stimulation            | exploratory            | University of Cambridge | behavior | N/A |
| Brain stimulation            | deep brain stimulation            | phase I                | Federal University of São Paulo | weight | NCT02297022 |
| Brain stimulation            | transcranial brain stimulation     | exploratory phase II   | University Kansas Medical Center Federal University of São Paulo Laval, Canada | hyperphagia; depression food cravings questionnaire; brain activity | NCT03602607 NCT0324906 NCT02758262 |

Examples of therapeutics in development not yet studied in PWS: Pitolisant (Wakix), ZGN-1061. Examples of therapeutics in PWS clinical studies but not controlled trials: ketogenic diet, tofogliflozin (SGLT2 inhibitor), metformin, naltrexone/bupropion (Contrave), N-acetylcysteine, risperidone. N/A, not applicable; HQ-CT, Hyperphagia Questionnaire for Clinical Trials; QOL, quality of life.
that are properly scaled to human doses, rather than dosing to maximum tolerability in the animal\textsuperscript{156}. Additional guidelines may be required for specific types of studies (e.g., mouse metabolism\textsuperscript{157} or behavior\textsuperscript{158}).

The second lesson, “know your animals,” was driven home during studies of amyotrophic lateral sclerosis (motor neuron disease, ALS) models in which human trials of tested compounds failed to replicate findings from the animal models\textsuperscript{159}. In mouse models of PWS, factors such as the pulsatile nature of growth hormone release, the frequent small meals consumed, and the fact that mice are quadrupedal complicate interpretation in studies of endocrine function, appetite control, and scoliosis, respectively. The small size and low fat mass of Snord116\textsuperscript{tm1.1Uta} and Del(7Ipw-Snord116)\textsuperscript{tm1Jbro} adult mutant mice and the novelty-induced anorexia described in Mage\textsuperscript{2tm1Stw} mice are examples of how mouse models may not completely capture phenotypes described in patients. It is important to use a model that actually demonstrates the endophenotype that is targeted by the therapeutic. The use of a decision flowchart that asks whether functional or surrogate endpoints can be measured in a particular model, and whether the mode of action of the therapeutic is understood, is recommended\textsuperscript{160}. Great care must be taken not to anthropomorphize results from animal models, but instead to interpret results within context of the physiology of the species and extrapolate the mechanism to support the utility (or not) of a particular intervention in clinical studies.

The third, and perhaps most difficult lesson, is that preclinical studies should ideally be performed in the context of, and with the support of, clinical trialists and adequate funding. Interactions between clinical and preclinical trialists would be facilitated if preclinical researchers could make an argument that a positive result from a preclinical study would either accelerate translation into clinical practice or would elucidate a mechanism of action thus accelerating the development of related therapies. Many promising therapeutics (e.g., beloranib) have never been tested in animal models of PWS (Table 1), and even compounds that have been tested in animal models (Table 1) have typically only been examined in one genetic model. Preclinical research is much more expensive than research designed to understand disease pathology or therapeutic mechanism of action in an animal model. The inclusion of both sexes, larger cohorts, different doses, and testing at different ages have a multiplicative effect on research budgets. The favored strategy to reduce costs, moving go/no-go decisions as early as possible in the pipeline,\textsuperscript{159} is difficult in preclinical research, where the majority of the financial investment (breeding of large cohorts) takes place very early in the experimental plan. A central registry for PWS preclinical trials that includes detailed methods would also facilitate the reproduction of promising results by other investigators.

We have reached an inflection point in preclinical studies of therapeutics in animal models of PWS. Increased investment in the preclinical arena is likely to accelerate the entry of compounds into a therapeutic pipeline and facilitate progress through the long clinical trials process. It is imperative that investigators preparing for preclinical studies learn from experiences in other rare disorders with neurological, muscular, endocrine, and other relevant issues by improving study design and reporting and by carefully choosing models and endpoints to maximally benefit individuals living with PWS.

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