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Review

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.

Prader-Willi syndrome (PWS) is a neurodevelopmental disorder caused by loss of expression of paternally expressed, imprinted genes on chromosome 15q11-q13.[1,2] Infants with PWS typically exhibit hypotonia and developmental delay, while older children and adults have intellectual impairment with behavioral abnormalities and neuropsychiatric symptoms, endocrine dysfunction with growth hormone deficiency, unrelenting hunger (hyperphagia) that can lead to obesity, and musculoskeletal and neurological abnormalities. Until recently, pharmacological treatments for PWS were limited to symptomatic therapies, such as hormone replacement therapy and psychiatric medications.[1] Behavioral and physical therapies also improve outcomes in people with PWS. However, there are no pharmacological therapies approved to treat symptoms that most affect daily activities including hyperphagia and emotional reactivity.

The number of clinical studies and trials testing therapeutics (compounds or procedures) to prevent or treat PWS symptoms has increased in recent years. To be used in clinical studies, these therapeutics have met minimum standards for safety in the general population but have not necessarily been tested for safety or efficacy in the PWS population. Animal models can be used to guide decision making about priorities for clinical trials and can provide information about potential efficacy of a compound within the context of a specific genetic disease background. Two reviews in 2013 summarized how mice carrying targeted mutations in PWS genes recapitulate many core phenotypes of PWS.[3,4] At that time, only two original research studies had attempted to rescue endophenotypes in mouse models of PWS. Both studies examined bioactive peptides, testing responses to either oxytocin[5] or melanotan II[6] in gene-targeted mice (Table 1). From 2014 to 2018, 15 additional studies used gene-targeted mouse lines to test the effects of pharmacological agents, surgical procedures, and environmental temperature on various phenotypes (Table 1). Here, we summarize how animal models contribute to progress toward effective therapeutics for PWS. We discuss obstacles to treatment in PWS that are typical of rare disorders, such as recapitulation of phenotypes in preclinical models. Finally, we discuss how careful design and reporting of preclinical studies can stimulate progress of therapeutics for 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).[2] 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 (*NPAP1*) 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

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Table 1. Interventional Trials in Mouse Models of PWS

Strain	Treatment	Treatment Administration	Note	Outcome	Group Size (M/F)	Age	Reference	Title
Magel2$^{tm1.1Mus}$	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
Magel2$^{tm1.1Mus}$	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
Magel2$^{tm1.1Mus}$	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
Magel2^{tm1Stw}	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
Magel2^{tm1Stw}	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
Magel2^{tm1Stw}	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
Magel2^{tm1Stw}	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
Magel2^{tm1Stw}	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
Magel2^{tm1Stw}	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
Magel2^{tm1Stw}	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
Snord116$^{tm1.1Uta}$	[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 anorexic effect of GHS-R inhibitors and exenatide in male Snord116 deletion mouse model for PWS
Snord116$^{tm1.1Uta}$	SPA	i.p. daily, 4.5 μmol/kg, 6 days	m	food intake	n = 8, 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

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Table 1. *Continued*

Strain	Treatment	Treatment Administration	Note	Outcome	Group Size (M/F)	Age	Reference	Title
Snord116$^{tm1.1Uta}$	YIL-781	i.p. daily, 134 μmol/kg, 6 days	n	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$^{tm1.1Uta}$	exenatide	i.p. twice daily, 24 μg/kg, 17 days	o	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$^{tm1.1Uta}$	GHSR agonist HM01	s.c. daily, 30 μg/g, 14 days	p	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$^{tm1.1Uta}$	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	Caralluma fimbriata extract activity involves the 5-HT2c receptor in PWS Snord116 deletion mouse model
Snord116$^{tm1.1Uta}$	thermoneutral (30°C)	16 weeks	r	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^{tm2Cbr} (PWS-ICdel)	thermoneutral (30°C)	9 weeks	s	food intake, fat mass, weight gain	n = 5–6, M and F	6–15 months	118	Paradoxical leanness in the imprinting-center deletion mouse model for PWS
Snrpn^{tm2Cbr} (PWS-ICdel)	WAY-161503	s.c. single injection, 3 mg/kg or 10 mg/kg	t	postfast refeeding	n = 12–14, M and F	adult	122	Increased alternate splicing of Htr2c in a mouse model for Prader-Willi syndrome leads disruption of 5HT2C receptor mediated appetite
Del(7Ube3a-Snrpn)1Alb	UNC0642	i.p. daily P7–P12, 2.5 mg/kg	u	% survival, body weight	n = 6–27, M and F	7–90 days	123	Targeting the histone methyltransferase G9a activates imprinted genes and improves survival of a mouse model of PWS

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.
[a]Rescued the death rate of Magel2 mice with a single injection of oxytocin 3–5 h after birth.
[b]Daily oxyocin injections in the first 7 postnatal days increased survival and prevented social and learning deficits in Magel2 adult male mice.
[c]Reversed social recognition deficits in Magel2 mice despite a temporary sedative effect.
[d]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 refeeding in control mice. This effect was no longer present by 4 h.
[e]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.
[f]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.
[g]Reversed obesity, reduced hyperphagia, increased total energy expenditure and voluntary activity, food intake and carbohydrate intake, and improved metabolic outcomes in obese Magel2 mice.
[h]Reduced food intake in Magel2 mice, compared to vehicle. Effect attributed to decreased meal size and accompanying increase in satiety ratio (postmeal interval/meal size).
[i]Decreased fat mass, increased percent lean mass, and eliminated hyperglycemia in male and female diet-induced obese mice. Both WT and Magel2 had improved endurance.
[j]Restored bone density and bone mass, decreased bone resorption, and increased bone formation.
[k]Daily fluoxetine treatment of Ndn pups from P5–P15 suppressed their respiratory deficits.
[l]No suppression of food intake in WT or Snord116 mice.
[m]No suppression of food intake in WT or Snord116 mice.
[n]Reduced food intake only on day 1 in WT and Snord116 mice.
[o]Food intakes for WT and Snord116 mice were reduced to ~84%.
[p]GHSR, growth hormone secretagogue receptor. Reduced body weight and length in male Snord116 pups, reduced mortality in Snord116 pups.
[q]Differences between strains in 4-h food intake after stimulation of appetite with agents. Sexes not separately analyzed.
[r]Normalized low BMD and BMC, high energy expenditure and low physical activity, but not low body weight, in Snord116 mice.
[s]Increased BAT mass in WT and ICdel males but failed to induce a corresponding elevation in WAT mass. Proportionate hyperphagia in ICdel males abolished at thermoneutrality.
[t]Reduced food consumption in WT but not PWS-ICdel mice compared to vehicle.
[u]Improved survival and increased growth of Del(7Ube3a-Snrpn)1Alb neonatal mice, 2/60 survived to P90.

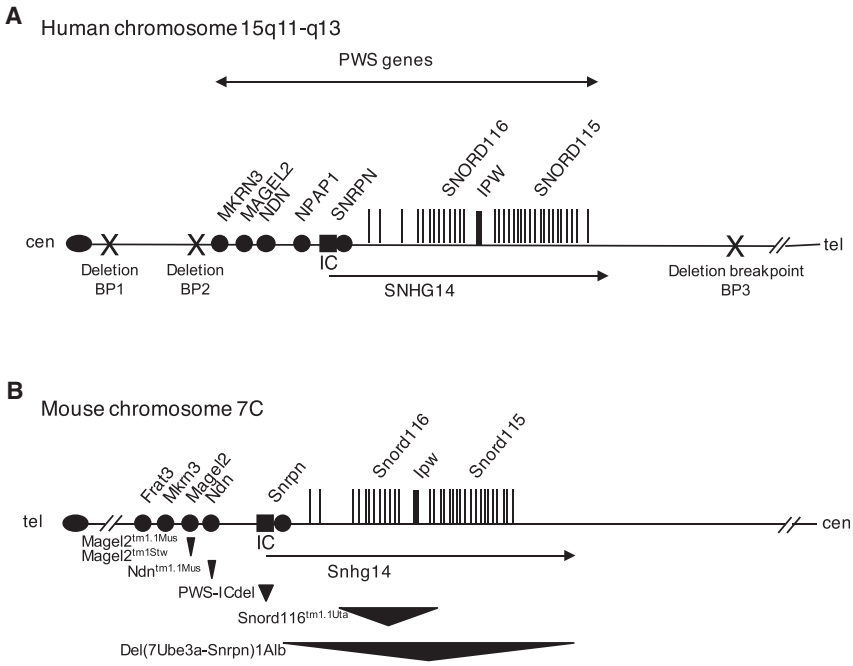

Figure 1. Genes Implicated in Prader-Willi Syndrome
(A) Paternally expressed, imprinted genes located within the PWS deletion region are indicated on a genomic map of human chromosome 15q11-q13. Protein coding genes and non-coding RNAs are indicated as circles and vertical lines, respectively. Common 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 interventional 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.

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.[7–9] 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.[10–12] *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.[9,12–14] For example, MAGEL2 interacts with TRIM27 and USP7 to control the activity of the WASH complex, mediating endosomal actin assembly and protein recycling.[12,13] *MAGEL2* also facilitates trafficking of cell-surface receptors,[13] including regulation of leptin receptors through interactions with the E3 ubiquitin ligase RNF41 and the deubiquitinase USP8.[14,15]

Consistent with the idea that loss of *MAGEL2* function plays a major role in the PWS phenotype, *de novo* or inherited protein truncating mutations in *MAGEL2* alone cause Schaaf-Yang syndrome (SHFYNG; OMIM: 615547).[16–24] Phenotypes in people with Schaaf-Yang syndrome overlap considerably with PWS phenotypes, including hypotonia, endocrine dysfunction, hypogonadism, developmental delay, intellectual disability with autism spectrum disorder, and maladaptive behavior.[25,26]

Mouse Models of Magel2 Deficiency

Two mouse models of *Magel2* deficiency have been developed.[3] The Magel2^{tm1Stw} mouse line carries a lacZ insertion that replaces the C-terminal domain of the *Magel2* open reading frame, including the MHD (JAX stock: 009062[11,27]). The Magel2$^{tm1.1Mus}$ line carries a deletion of the *Magel2* promoter and most of the open reading frame.[5] Magel2^{tm1Stw} 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.[3,4] Consistent with their excess fat mass, adult Magel2^{tm1Stw} mutant mice are insensitive to the anorexigenic effects of injected leptin hormone.[6] Moreover, their hypothalamic POMC neurons do not depolarize in response to leptin.[6] Magel2$^{tm1.1Mus}$ mutant mice demonstrated suckling defects causing slow growth or lethality in pups.[5]

Since 2012, additional phenotypes have been uncovered in *Magel2* mutant mice. Magel2^{tm1Stw} 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 social novelty.[28] Magel2$^{tm1.1Mus}$ male mutant mice exhibit deficits in social recognition and social interaction and reduced ability to learn.[29] Sleep deficits in Magel2^{tm1Stw} mutant mice have also been reported.[30] While adult Magel2^{tm1Stw} mutant mice demonstrate physiological leptin resistance, younger Magel2^{tm1Stw} mutant mice are leptin sensitive.[15] Consistent with this phenotype, a progressive postnatal decline in leptin sensitivity was detected in POMC neurons in live tissue slices of the arcuate nucleus of the hypothalamus.[15] Magel2^{tm1Stw} mutant mice exhibit impaired development and

function of hypothalamic anorexigenic circuits.[31,32] As well, Magel2^{tm1Stw} mutant mice demonstrate dopamine pathway imbalances[33] and histological and functional muscle impairment, including a progressive reduction in limb strength and endurance with age.[10] Tissues from Magel2^{tm1Stw} 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.[10] 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 Magel2^{tm1Stw} mutant mice,[14] consistent with a role for *MAGEL2* in membrane protein recycling.[9] 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 *Magel2* mutant mice and individuals in PWS all support the hypothesis that loss of MAGEL2 makes a major contribution to PWS.

Interventional Studies in Magel2 Mutant Mice

Nine studies have examined the effect of therapeutic interventions in *Magel2* mutant mice (Table 1). A postnatal injection of oxytocin improved survival of Magel2$^{tm1.1Mus}$ mutant mice, which otherwise have an early postnatal mortality rate of about 50%.[5] In a follow-up study, early postnatal treatment of Magel2$^{tm1.1Mus}$ mutant mice with oxytocin prevented the social and learning deficits that adult Magel2$^{tm1.1Mus}$ mutant mice would otherwise display.[29] Administration of MT-II, a melanocortin 4 receptor agonist, decreased food intake in Magel2^{tm1Stw} mutant mice to a greater extent than in WT controls.[6] In a follow-up study, Magel2^{tm1Stw} 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.[34] Magel2^{tm1Stw} mutant mice responded to bariatric surgery (sleeve gastrectomy) with loss of fat mass and improved metabolic parameters.[35] Administration of JD5037, which targets the endocannabinoid and CB1 receptor system, reduced fat mass in Magel2^{tm1Stw} mutant mice.[36] Magel2^{tm1Stw} 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.[37] Magel2^{tm1Stw} mutant mice have high fat mass and reduced endurance,[10] phenotypes that were ameliorated by chronic diazoxide treatment.[38] Magel2^{tm1Stw} mice have dysfunctional oleoylethanolamide signaling, and intraperitoneal administration of oleoylethanolamide significantly reduced food intake on fasting and refeeding.[39] Overall, these results suggest that *Magel2* 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.[40] 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.[41,42] 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$^{tm1.1Mus}$,[43] Ndn^{tm1Alb},[44] Ndn^{tm1Ky} (repository RBRC02316[45]), and Ndn^{tm2Stw} (JAX stock: 009089[46]).[3] All four lines carry deletions within the coding region, and the promoter is also deleted in one of the lines (Ndn$^{tm1.1Mus\ 5}$). 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.[43,45–62] Necdin regulates preadipocyte proliferation in developing adipose tissues, and Ndn^{tm1Ky} become obese with adipocyte hyperplasia when fed a high-fat diet.[63,64] 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.[49,60] Interestingly, necdin is important for normal hematopoiesis, although hematopoietic defects are not described in PWS.[65,66] 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.[53,67] Necdin also modulates osteogenic cell differentiation by regulating two other genes, *Dlx5* and *Maged1*.[68]

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.[69–71] Relevant to 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$^{tm1.1Mus}$ mice.[72] Necdin regulates the quiescence and response to genotoxic stress of hematopoietic stem and progenitor cells, as demonstrated using Ndn^{tm2Stw} mutant mice.[73–77] Necdin also protects neurons against mitochondrial insults by promoting mitochondrial biogenesis, in a study that used the Ndn^{tm1Ky} mice.[78] Necdin controls epidermal growth factor receptor signaling linked to astrocyte differentiation,[79] interacts with the RING finger protein Bmi1 to control the proliferation of neural precursor cells in the neocortex,[80] and modulates the thyroid axis.[81] Along with MAGEL2, necdin facilitates leptin receptor recycling, as shown in cultured cells and in mouse brain tissue.[14] Lastly, functional interactions between necdin and other proteins have been uncovered,

including interactions with the alpha subunit of the guanine nucleotide-binding protein G0[82] 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 animals 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 Ndn$^{tm1.1Mus}$ mutant 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.[71]

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.[94] The deletions within *SNHG14*/*SNORD116* cluster could modify the expression of *MAGEL2* through long-range chromatin interactions.[95] This would explain how microdeletions 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)-Snord116$^{tm1.1Uta}$/J[96] and Del(7Ipw-Snord116)^{tm1Jbro}.[97] Other transcripts, such as the murine equivalents of *SNHG14* and *IPW*,[98] 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 Snord116$^{tm1.1Uta}$ 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 Snord116$^{tm1.1Uta}$ mice in one study.[104] Another study of the same mice revealed no differences in hypothalamic *Pcsk1* expression in either fed or fasted states.[103] Interestingly, the hypothalamus-specific reintroduction of *Snord116* into Snord116$^{tm1.1Uta}$ mutant mice increased energy expenditure.[105,106] Adult-onset deletion of *Snord116* in conditional (floxed) Snord116^{tm1Uta} 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.[103] These seemingly contradictory results have yet to be resolved. In Snord116$^{tm1.1Uta}$ 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). Snord116$^{tm1.1Uta}$ mutant mice had an abnormal response to the anorexic 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 Snord116$^{tm1.1Uta}$ mutant mice, and the effect on 4-h food intake was measured after 9 weeks of treatment. CFE appeared to decrease food intake in Snord116$^{tm1.1Uta}$ mutant 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 Snord116$^{tm1.1Uta}$ mutant mice at a thermoneutral temperature (30°C), instead of the 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^{tm2Cbr}/J). Surviving PWS-ICdel have reduced locomotor activity and cognitive deficits.[117] 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.[118] After an overnight fast, the PWS-ICdel mice consumed more food than WT in the first 30 min of refeeding,[119] perhaps because their small size increases their energy needs at ambient temperature, or because of an underlying mitochondrial dysfunction.[120] Another group generated a line of mice (Del(7Ube3a-Snrpn)^{1Alb}) carrying a targeted deletion from *Snrpn* to *Ube3a*, but not including the IC.[121] Similar to the Snord116 mice, Del(7Ube3a-Snrpn)^{1Alb} mutant mice have severe growth retardation, hypotonia, and high rates of lethality before weaning.[121] 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.[118] 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-HT_{2C}R-specific agonist, WAY-161503, was investigated in PWS-ICdel mutant mice.[122] 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 snoRNA cluster Snord116, and improved survival of Del(7Ube3a-Snrpn)^{1Alb}) mice.[123] 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.[123]

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

MKRN3 (Makorin ring finger protein 3) is widely expressed throughout the body.[124] *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.[125] 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 nucleoporin gene POM121.[126] *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 *p* (pink-eyed dilution) gene[127] 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.[128] Likewise, some individuals with PWS have larger deletions (type I deletions) that include non-imprinted genes (*TUBGCP5*, *NIPA1*, *NIPA2*, and *CYFIP1*) proximal to the imprinted gene cluster.[129] 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.[130,131] 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.[132–150] A trial of beloranib, a methionine aminopeptidase 2 (MetAP2) inhibitor that promotes loss of fat mass, was terminated in 2016 after adverse events involving excess blood clot formation,[151] while other MetAP2 compounds are in development.[152] An open label 6-month trial of exenatide (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.[153] 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.[130]). These include GLWL-01 for treatment of hyperphagia (NCT03274856), cannabidiol oral solution for treatment of hyperphagia-related behavior and reduction of body weight (NCT02844933), co-administration of tesofensine and metoprolol for reduction of body weight (tesomet, NCT03149445), 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.

Table 2. Categories of Compounds Tested in Both Preclinical (Mouse) Models of PWS and in Humans with PWS

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) FDA approved	Magel2: [5] Magel2: [29]	phase II phase III, [133,135,136,149]	many sites	infant suckling; food intake; hyperphagia; behavior; social behavior	NCT03197662 NCT02205034 NCT02013258 EudraCT: 2010-022370-14 EudraCT: 2017-003423-30
	carbetocin/FE 992097 EU approved	not trialed	phase II phase III, [150]	Ferring Levo Therapeutics	hyperphagia; behavior	NCT01968187
K^+-ATP channel agonist	diazoxide FDA approved	Magel2: [38]	not trialed			
	DCCR/extended release diazoxide choline investigational	not trialed	phase II phase III	Essentialis Soleno Therapeutics	hyperphagia; REE	NCT02034071 NCT02893618 NCT03440814
Melanocortin 4 receptor agonists	MT-II/Melanotan 2 not approved	Magel2: [6]	not trialed			
	RM-493 or setmelanotide investigational	Magel2: [34]	phase II	Rhythm	weight loss; hyperphagia-related behavior	NCT02311673
GLP-1 receptor agonists	exenatide (Byetta) FDA approved	Snord116: [113]	exploratory, open label phase II [153,166]	Children's Hospital Los Angeles Aintree University Hospital	weight; metabolism ghrelin levels	NCT01444898 EudraCT: 2010-023179-25
	exenatide extended release (Bydureon)	not trialed	phase III	Garvan Foundation, Australia	gastric emptying	ACTRN12616000710426
	liraglutide (Saxenda)	not trialed	phase III	Novo Nordisk	BMI; hyperphagia; metabolism	NCT02527200 EudraCT: 2014-004415-37
Cannabinoids	oleoyl α-methyl serine (HU-671) investigational	Magel2: [37]				
	JD5037 or SLV-319 (ibipinabant) research only	Magel2: [36]				
	OEA (oleoylethanolamide) supplement	Magel2: [39]				
	cannabidiol (CBD) oral solution Under review by FDA		phase II	Insys Therapeutics	hyperphagi, weight	NCT02844933 NCT03458416
	rimonabant (Acomplia) withdrawn		phase II [167]	Cornell Medical College Karolinska University Hospital	obesity	NCT00603109 EudraCT: 2007-006305-25
Ghrelin analog	ghrelin receptor agonist HM01	Snord116: [114]				
	livoletide (AZP-531) GLWL 01		phase IIa [168] phase II phase II	Alize Millendo Therapeutics GLWL Research	blood glucose levels; weight hyperphagia hyperphagia, behavior	EudraCT: 2014-001670-34 NCT03790865 NCT03274856
Natural supplement	cactus extract from *Caralluma fimbriata*	Snord116: [165]				
	cactus extract from *Caralluma fimbriata*		phase I [169]	Victoria University, Australia	hyperphagia; behavior	ACTRN12611000334909

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 FDA approved	prescribed as needed	Karolinska University Hospital; Novo Nordisk	body composition; linear growth; bone mineral density; cognitive and adaptive function	NCT00372125 NCT00705172
Stimulant	modafinil FDA approved	case series, open label [161]	Hôpital Purpan, Toulouse, France	sleepiness	N/A
Synthetic somatostatin	octreotide FDA approved	phase II [162]	British Columbia's Children's Hospital	BMI; appetite; behavior; ghrelin concentration	NCT00175305
Anticonvulsant	topiramate FDA approved	phase II phase III	University of Florida-Brain Institute Hopitaux de Paris	self-injurious behavior eating disorders; self-mutilation; irritability and impulsivity; metabolic status	NCT00065923 NCT02810483
Aromatase Inhibitor	anastrozole FDA approved	phase II	Hôpital Armand Trousseau, Paris	bone maturation related to pathological adrenarche	NCT01520467
Serotonin-noradrenaline-dopamine reuptake inhibitor/beta-blocker	tesomet (tesofensine and metoprolol) Investigational/FDA approved	phase II	Saniona	body weight	NCT03149445 EudraCT:2016-003694-18
Ghrelin pathway	GLWL-01 investigational	phase II	GLWL Research	hyperphagia, HQ-CT score	NCT03274856
Methionine aminopeptidase 2 inhibitor	beloranib not approved	phase II phase III [151]	Zafgen	HQ-CT; weight; fat and lean; QOL	NCT01818921 NCT02179151 EudraCT:2015-000660-33
Probiotic	*Bifidobacterium lactis* B94 (probiotic) supplement	phase II	University of Florida, Gainesville	stool frequency	NCT03277157
Brain stimulation	vagal nerve stimulation FDA approved	exploratory	University of Cambridge [163]	behavior	N/A
Brain stimulation	deep brain stimulation FDA approved	phase I	Federal University of São Paulo	weight	NCT02297022
Brain stimulation	transcranial brain stimulation FDA approved	exploratory [164] phase II	University Kansas Medical Center Federal University of São Paulo Laval, Canada	hyperphagia; depression food cravings questionnaire; brain activity	NCT01863017 NCT03324906 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.

How Can We Improve Translation to Clinical Trials and Reduce 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."[154] Important recommendations have been developed to address issues of translatability of preclinical studies to clinical trials (e.g., ARRIVE guidelines[155]). 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.[155] Where possible, preclinical studies should use doses

that are properly scaled to human doses, rather than dosing to maximum tolerability in the animal[156]. Additional guidelines may be required for specific types of studies (e.g., mouse metabolism[157] or behavior[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.[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$^{tm1.1Uta}$ and Del(7Ipw-Snord116)^{tm1Jbro} adult mutant mice and the novelty-induced anorexia described in Magel2^{tm1Stw} 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.[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 3), 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,[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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