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Commentary

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Hypothalamic loss of *Snord116* and Prader-Willi syndrome hyperphagia: the buck stops here?

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Hyperphagia and obesity are the best-known manifestations of Prader-Willi syndrome (PWS) and are responsible for most of the overall morbidity and mortality associated with the disease. Yet these PWS symptoms remain poorly understood and without effective pharmacologic therapies. Mouse models attempting to recapitulate both the genetic alterations and marked hyperphagia plus obesity of PWS have been enigmatic, leading to skepticism about the use of mouse models to investigate PWS. In this issue of the *JCI*, Polex-Wolf and colleagues challenge the skeptics by successfully inducing hyperphagia following bilateral mediobasal hypothalamic deletion of the *Snord116* gene from adult mice. Obesity also resulted, although only in a subset of mice. While this approach represents an exciting advance, highlighting a pathologic effect of loss of mediobasal hypothalamic *Snord116* expression on the development of PWS's hallmark symptoms, the variability in the body-weight and body composition responses to this site-selective gene deletion raises several questions.

Hyperphagia in Prader-Willi syndrome

Prader-Willi syndrome (PWS) is a genetic disorder affecting multiple organ systems. Hypotonia is particularly prominent in the neonatal period, causing lethargy and a poor suck reflex, which in turn contribute to feeding difficulty and failure to thrive, often necessitating feeding-tube placement. Hypotonia in the respiratory musculature contributes to recurrent respiratory infections, which are another source of significant childhood morbidity and mortality (1–3). Individuals with PWS also often experience learning disabilities, disruptive behavioral problems, mental health issues, and a high pain threshold (1, 4, 5). Furthermore, disorders of several endocrine systems are usually present, including, most prominently, growth-hormone deficiency (1, 6).

The preeminent symptom of PWS is hyperphagia, which develops at a median age of 8 years, is fueled by an insatiable appetite, and is manifested by a set of food-seeking behaviors that include binge eating, hoarding, pica, and food foraging, typically resulting in obesity despite careful monitoring (1, 6–9). This hyperphagia figures prominently in later childhood and into adulthood, contributing to most of the morbidity during that period and to early mortality, with most individuals with PWS not living past their early 40s (1). Earlier detection of the disorder, increased access for families to PWS resources and support groups, and the development of more inclusive, multidisciplinary treatment plans, which include growth-hormone replacement, have resulted in improvements in the overall management of PWS; however, the burden of disease stemming from hyperphagia remains high and without specific, highly efficacious therapeutic options — pharmacologically based or otherwise.

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Mouse models of PWS

The creation of genetically modified mouse models targeting the same chromosomal regions affected in humans with PWS represents one approach to gaining a better understanding of the biological basis for the hyperphagia of PWS and for the development and testing of potential treatments. Over thirty genetic mouse models of PWS have been generated by deleting fragments of mouse chromosome 7 that correspond to segments of human chromosome 15 associated with PWS (10). The sporadic loss of or failure to express one or more of the paternally expressed genes within the human chromosome 15 segment results in PWS (1, 10). Unfortunately, mice lacking the entire PWS domain homolog die soon after birth (10). While several mouse models lacking individual genes within the PWS region have better survivability, they often manifest only a subset of the phenotypes that characterize PWS (10, 11).

One such single-gene deletion model of PWS is the *Magel2*-null mouse. This line carries a paternally inherited *lacZ* gene in place of the *Magel2* coding sequence and thus lacks the PWS-implicated gene *Magel2*. Moreover, this model recapitulates several PWS-associated phenotypes, including hypotonia, increased adiposity, and hyperphagia. In particular, male and female *Magel2*-null mice exhibit increased fat mass by 18 weeks of age whether fed regular chow or high-fat diet (HFD) (12, 13). Although these animals have been characterized as obese, perhaps they would be more accurately characterized as having increased adiposity instead of obesity per se, as increases in body weight, when observed in females on HFD, remain slight (12, 13). Similarly to what occurs in PWS, *Magel2*-null mice show reduced energy expenditure, reduced lean mass in females, and decreased muscle tone and strength (12, 13). Importantly, both male and female *Magel2*-null mice exhibit hyperphagia when fed HFD (12, 13).

Another single-gene deletion model of PWS is the *Snord116*$^{+/-P}$ mouse, with a congenital paternal *Snord116* deletion (14). Of note, a human subject with a microdeletion limited to the *SNORD116* gene cluster displayed many similarities, including hyperphagia, excessive weight gain, and obesity beginning in early childhood, observed in PWS subjects with more common chromosomal deletions of the entire PWS critical region (15). Of the many key PWS features recapitulated within *Snord116*$^{+/-P}$ mice, growth delay marked by reduced body weight and reduced body length and an impaired growth-hormone pathway seem most prominent, while frank hypotonia is not present (14, 16, 17). As regards to the eating and body-weight phenotype of adults with PWS, some studies have described *Snord116*$^{+/-P}$ mice as exhibiting slight hyperphagia, but only when their body weights are taken into consideration (14, 16, 17). In other words, while the lighter *Snord116*$^{+/-P}$ mice have been shown to eat more food per body weight and demonstrate higher body-weight gain, individual *Snord116*$^{+/-P}$ mice do not eat more food than the heavier WT mice. Prolonged eating bouts, which suggest impaired satiation, and increased food intake during a fast-refeed protocol also have been noted in *Snord116*$^{+/-P}$ mice. That said, *Snord116*$^{+/-P}$ mice do not become obese (14), nor do mice with biallelic *Snord116* deletion become obese (18, 19). Importantly, as opposed to what occurs in PWS, which is associated with reduced energy expenditure, adult *Snord116*$^{+/-P}$ mice and adult mice with biallelic *Snord116* deletion display increased energy expenditure when housed at room temperature, which is speculated to block the development of obesity in the mice (14, 18, 19), as likely does the initial growth delay.

Hyperphagia plus obesity in a PWS mouse model

The current work of Polex-Wolf and colleagues (20) moves to further investigate the role of *Snord116* loss in the development of PWS. The authors took an existing floxed *Snord116* mouse model (*Snord116*^{fl}) in which *loxP* sites surround the *Snord116* locus (14) and introduced adeno-associated virus (AAV) expressing Cre recombinase bilaterally into the mediobasal hypothalamus, thereby selectively deleting *Snord116* expression within that region of the brain. Since these mice underwent the stereotaxic virus injections at 10 weeks of age, they developed without the prominent growth delay phenotype that characterizes *Snord116*$^{+/-P}$ mice. As compared with control mice receiving similar injections of AAV expressing GFP, mice correctly targeted with the Cre-expressing AAV exhibited hyperphagia when assessed 9 to 10 weeks after surgery using the analysis of covariance (ANCOVA) methodology, which corrects for body-weight differences. As an aside, Polex-Wolf and colleagues were unable to demonstrate hyperphagia in *Snord116*$^{+/-P}$ mice when the ANCOVA model was applied to the analysis. Notably, adult-onset mediobasal hypothalamic *Snord116* deletion also resulted in obesity — as characterized by a body-weight gain over 140% of presurgery weight and the development of increased fat mass by 10 weeks after surgery — in five out of twenty-one mice. No energy expenditure differences were noted between the Cre-exposed and GFP-exposed animals at six weeks after surgery, at which time body-weight curves of the two groups began to diverge. *Snord116* loss as a result of germline whole-body deletion in *Snord116*$^{+/-P}$ mice or germline selective deletion from neuropeptide Y neurons, including orexigenic neuropeptide Y neurons in the mediobasal hypothalamus (18, 19), caused growth delay with lower body weights. On the other hand, the approach of AAV-mediated mediobasal hypothalamic deletion of *Snord116* from adult animals, which necessarily bypasses the neonatal period, resulted in both frank hyperphagia and, in a subset of animals, frank obesity.

Questions raised

Why did hyperphagia plus frank obesity develop only in five of the twenty-one mice? As a group, all twenty-one mice were hyperphagic, and the subset of five mice that gained over 140% of their presurgery weights and developed marked increases in fat mass were not any more hyperphagic than the sixteen other correctly targeted mice. Notably, higher expression of hypothalamic *Socs3* was detected in the obese subset. As Polex-Wolf et al. remark, Socs3 affects hypothalamic signaling pathways downstream of leptin and, when overexpressed in proopiomelanocortin (POMC) neurons, increases body weight and adiposity (21–23). Thus, further investigations into a potential connection between Snord116 and Socs3 and a functional role of altered mediobasal hypothalamic Socs3 signaling in the development of hyperphagia and obesity associated with PWS seem warranted. Polex-Wolf and colleagues also discuss the possibility of polyphenism, in which disparate phenotypes occur despite underlying identical genotype and environmental conditions. Differential *Snord116* loss is another potential factor in the variable expression of obesity. In particular, while the extents of mediobasal hypothalamic Cre expression achieved were similar in the two groups of mice, the investigators did not specifically determine whether there were any differences in *Snord116* deletion between the subset that exhibited the more marked body-weight gain and the group that did not gain as much. A method that allows for a more uniform spatiotemporal targeting of Cre recombinase to the mediobasal hypothalamus or to specific mediobasal hypothalamic cell types would help reduce the potential for differential *Snord116* deletion between mice in future studies. Although less likely, given that the gene manipulations were all done in adult Snord116^{fl} mice, it might also be worthwhile to determine whether differences in original litter size, genotypic or sex makeup of littermates, or maternal nurturing may have influenced the body-weight phenotypes of the mice. Additionally, it would be of interest to examine the effect of viral-mediated mediobasal hypothalamic deletion of *Snord116* from adult female mice, as the Polex-Wolf et al. study focused on males and as previous work with *Magel2*-null mice revealed higher body weights in female mice (12, 13).

Notably, a related approach, involving adult-onset whole-body *Snord116* deletion, did not cause hyperphagia or obesity (24). Instead, these mice, which carried two Snord116^{fl} genes and a gene expressing a tamoxifen-inducible Cre recombinase under the control of the ubiquitin promoter, and in which the Cre-mediated Snord116 deletion was induced at eight weeks of age, did not exhibit body-weight or body composition phenotypes and unexpectedly exhibited decreased food intake (24). While the expected body-weight and food-intake changes may not have been

observed due to the partial (16%) reduction in hypothalamic expression of *Snord116* (24), one must also consider the possibility that differential loss of *Snord116* in other tissues may somehow counter the effects of its loss in the mediobasal hypothalamus.

Additionally, Polex-Wolf and colleagues did not detect differences in hypothalamic expression of *Pcsk1*, which encodes prohormone convertase 1/3, or *Nhlh2*, a transcription factor that regulates Pcsk1, in *Snord116*$^{+/-P}$ mice or in mice with viral-mediated mediobasal hypothalamic *Snord116* deletion. This is seemingly counter to the recent *JCI* study by Burnett and colleagues in which iPS cell–derived (iPSC-derived) neurons from human subjects with the most common genetic form of PWS or with a microdeletion of the *SNORD116* gene and two flanking genes were shown to display markedly downregulated expression of *PCSK1* and *NHLH2* (17). Reduced *Nhlh2* and/or *Pcsk1* expression also were observed in a number of tissues from *Snord116*$^{+/-P}$ mice, including the hypothalamus (17). Downregulation of these genes is predicted to restrict maturation of multiple hormones and neuropeptides, including several known to affect eating, body weight, and growth, throughout the body. Impaired prohormone processing of progrowth-hormone–releasing hormone, proinsulin, and proghrelin were all observed, and it was postulated that insufficient processing of mediobasal hypothalamic POMC or hypothalamic prooxytocin could contribute to the hyperphagia and obesity of PWS (17). Further exploration of the potential role of altered *Pcsk1* expression, including the contribution of timing, in the phenotypic manifestations of PWS is warranted, especially given the lack of changes in Pcsk*1* and *Nhlh2* expression in Polex-Wolf et al.

Final thoughts

Polex-Wolf and colleagues have generated a new preclinical model of PWS, with both hyperphagia and obesity as prominent phenotypes, at least in a subset of individuals. While the apparent unpredictability in obesity occurrence might limit the utility of this model for testing compounds to prevent obesity in PWS, the model may be useful to test the anorexigenic actions of potential drugs and, in the subset of mice that do develop obesity, their weight-reducing efficacy.

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