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Commentary

Prader-Willi syndrome (PWS) is a complex disorder that manifests with an array of phenotypes, such as hypotonia and difficulties in feeding during infancy and reduced energy expenditure, hyperphagia, and developmental delays later in life. While the genetic cause has long been known, it is still not clear how mutations at this locus produce this array of phenotypes. In this issue of the *JCI*, Burnett and colleagues used a comprehensive approach to gain insight into how PWS-associated mutations drive disease. Using neurons derived from PWS patient induced pluripotent stem cells (iPSCs) and mouse models, the authors provide evidence that neuroendocrine PWS-associated phenotypes may be linked to reduced expression of prohormone convertase 1 (PC1). While these compelling results support a critical role for PC1 deficiency in PWS, more work needs to be done to fully understand how and to what extent loss of this prohormone processing enzyme underlies disease manifestations in PWS patients.

Impaired prohormone processing: a grand unified theory for features of Prader-Willi syndrome?

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Prader-Willi syndrome: a complex disorder

Prader-Willi syndrome (PWS) is a congenital multisystem disorder that is characterized by neonatal hypotonia and feeding problems, as well as developmental delay, short stature, hypogonadotropic hypogonadism, decreased resting energy expenditure with hyperphagia, and severe obesity appearing in later childhood. The genetic locus responsible for PWS is located on a large maternally imprinted region of chromosome 15q11–q13 and has been known since the 1970s. Despite extensive research into this syndrome, the molecular mechanisms underlying the complex clinical phenotype are still largely unknown. The majority of PWS cases (70%–75%) are due to deletions on the paternally inherited chromosome 15, with a remainder of cases caused mainly by maternal uniparental disomy for region 15q11–q13 and rare cases of imprinting defects and balanced translocations. In recent years, patients have been identified who have microdeletions that encompass the small nucleolar RNA C/D box 116 cluster (*SNORD116*) on chromosome 15q11.2 and manifest as a phenotype that substantially overlaps with PWS, suggesting the possibility of a smaller, so-called PWS minimal deletion region (1–5). This genomic cluster encodes noncoding RNAs, including long noncoding RNAs (lncRNAs) and an array of small nucleolar RNAs (snoRNAs), the function and physiological targets of which are poorly characterized.

It has been challenging to gain a complete understanding of the relationship between genetic defects and the various phenotypic manifestations of PWS. For example, none of the existing PWS mouse models recapitulate the characteristic hyperphagia phenotype that leads to severe obesity. In this issue, Burnett and colleagues (6) make an effort to circumvent the challenges of accessing human neuronal tissue by creating induced pluripotent stem cell–derived (iPSC-derived) neurons from PWS patients with deletions that encompass *SNORD116*. Transcriptomic analysis of these iPSC-derived neurons revealed that *PCSK1*, which encodes prohormone convertase 1 (PC1), is one of the most downregulated genes in PWS-derived cells, raising the possibility of impaired prohormone processing. Moreover, mice lacking *Snord116* had decreased PC1 levels in the pancreas and hypothalamus, and these animals also exhibited a trend of decreased PC1 in the stomach compared with control mice. Concomitant increases in proinsulin, pro-growth hormone–releasing hormone (proGHRH), and proghrelin further suggest that these decreases in PC1 are physiologically relevant. In serum, the ratio of proinsulin to insulin was elevated in a PWS patient but not to the same level as that of a patient without functional PC1. Burnett and colleagues thus postulate that deficiencies in PC1, as a consequence of *SNORD116* deletion, drive the major neuroendocrine phenotypes of PWS. The gene encoding the transcription factor nescient helix loop helix 2 (*NHLH2*) was also among the most downregulated genes in iPSC-derived neurons from PWS patients. Mice lacking NHLH2 also show some PWS-like phenotypes, including impaired early growth and later obesity (7). NHLH2 binds to the promoter of *PCSK1* and enhances transcription, providing a possible additional link to impaired prohormone processing. However, it is likely that NHLH2 regulates transcription of many genes; therefore, it is possible that NHLH2 influences energy balance through its effects on the expression of genes other than *PCSK1*.

The current work by Burnett et al. highlights the strengths of a combined approach using human and mouse models to study a complex neuroendocrine disorder and adds

▶ Related Article: p. 293

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to a growing literature using iPSCs to investigate the molecular etiology of PWS (8, 9). PC1 deficiency represents an intriguing potential mechanism that links many of the diverse phenotypes observed in PWS. For example, patients with inactivating mutations of *PCSK1* manifest a clinical phenotype of syndromic obesity with some parallels to PWS, and one report has suggested that a subset of PWS patients may have reduced expression of a closely related proprotein convertase, PC2, in the hypothalamic paraventricular and supraoptic nuclei (10). Genetically modified mice lacking *Nhlh2* or *Pcsk1* further mirror aspects of the natural history of PWS in humans. Homozygous deletions of *Pcsk1* in the mouse result in a high rate of early lethality and a failure to thrive, while hypomorphic *Pcsk1* mice develop obesity in adulthood (11). *Nhlh2* deletion mice display hypogonadism and adult-onset obesity, potentially driven by defects in energy expenditure (7, 12).

Elevated levels of ghrelin, including the active acylated form, have been repeatedly demonstrated in patients with PWS (13–15), with an obvious implication that hyperghrelinemia might be a mechanism that drives the hyperphagia that is characteristic of PWS. However, hyperghrelinemia appears in infancy well before the onset of hyperphagia (16, 17), and ghrelin-reducing agents do not alleviate hyperphagia in individuals with PWS (18). Recent studies found no elevation in total ghrelin in *Snord116*$^{-/-}$ mice (19). Burnett et al. pose an interesting hypothesis that the high levels of circulating ghrelin reported in human PWS could possibly be due to cross-reactivity of most assays with proghrelin. A comprehensive analysis of the specific products of the proghrelin gene in human PWS patients and controls is urgently needed.

Remaining questions and future directions

Can a deficiency of PC1 explain all of the endocrine and metabolic features of PWS? While there is some phenotypic overlap between *PCSK1*-deficient humans and those with PWS, there are also many manifestations that are different (see ref. 11 for a review of PC1 deficiency). Impaired proopiomelanocortin (POMC) processing in *PCSK1*-deficient patients results in pale skin, reddish hair, as well as low cortisol and adrenocorticotropic hormone (ACTH) levels — phenotypes that are not characteristic of PWS. Diabetes insipidus is also common in *PCSK1*-deficient patients but not often observed in PWS. Growth hormone deficiency is a highly prevalent characteristic of PWS but has only been reported in a limited subset of *PCSK1*-deficient patients. Several other differences appear when examining the natural history of these disorders. *PCSK1*-deficient patients present with severe neonatal malabsorptive diarrhea, while in PWS, infancy is characterized by a failure to thrive that is linked to hypotonia and feeding difficulties. Burnett et al. state "Our findings suggest that the major neuroendocrine features of PWS are due to PC1 deficiency." While this work has opened up an important new investigative window on possible mechanisms of the neuroendocrine features of PWS, it is perhaps premature to conclude that PC1 deficiency is the sole explanation for these.

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