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Leptin signaling defects in a mouse model of Prader-Willi syndrome

An orphan genetic obesity syndrome no more?

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Prader-Willi syndrome (PWS) is a rare (~1 in 12,000) genetic disorder that involves at least six genes on chromosome 15q11–q13. Children with PWS not only rapidly gain weight and become severely obese because of reduced voluntary activity and increased food intake, but also exhibit growth hormone deficiency, excessive daytime sleepiness, endocrine dysregulation and infertility. These phenotypes suggest dysfunction of the hypothalamus, the brain region that regulates short- and long-term energy balance and other body functions. The physiological basis for obesity in children with PWS has eluded researchers for decades. Mercer et al. now demonstrate that *Magel2*, the murine ortholog of one of the PWS genes, is a component of the hypothalamic leptin-melanocortin pathway that is critical for energy balance. Most interestingly, disruptions of other components of this pathway cause obesity in both mice and humans, suggesting a mechanistic link between PWS and other rare genetic forms of severe childhood-onset obesity.

Obesity and obesity-associated complications are a leading cause of morbidity, mortality and excess health care costs.[1] While the heritability of body mass index in adults is estimated at 40–70%,[2] genetic factors contribute to over 80% of weight variation in children and adolescents.[3] Many obesity susceptibility genes act in the central nervous system, interacting with each other and with an environment that provides easy access to cheap, calorically dense and highly palatable food.[4-6] Studies that identify and characterize novel obesity genes and pathways have already been shown to have great potential to help us understand, prevent and treat obesity.

Most childhood-onset, severe, syndromic or heritable forms of obesity are caused by rare mutations in genes important in energy balance control circuits in the hypothalamus (**Fig. 1**).[7-12] This small region of the brain coordinates the nervous and endocrine systems to regulate energy balance and other homeostatic activities.[13] Both rare mutations and common variants have been found in genes encoding proteins that are involved in the neural responses to leptin, a key hormone produced by adipose tissue. Mutations in these genes profoundly affect body weight and are not compensated for by other genes or pathways, highlighting their physiological importance. Further, leptin resistance is a hallmark of diet-induced obesity.[14] The recent report by Mercer et al. demonstrating that loss of a protein named Magel2 impairs leptin signaling in the hypothalamus introduces a new member to the cast of characters essential for energy homeostasis.[15] *Magel2* is the murine ortholog of *MAGEL2*,[16-23] one of the genes that is inactivated in the orphan genetic obesity disorder Prader-Willi syndrome (PWS).[21,24-26] This raises a question about this rare and poorly understood disease: is PWS still an orphan genetic obesity disorder or is it too caused by a defect in hypothalamic leptin signaling?

Severely obese children with mutations in the genes encoding leptin, its receptor and downstream signaling components have intractable feelings of hunger (hyperphagia) and aggressive behavior around

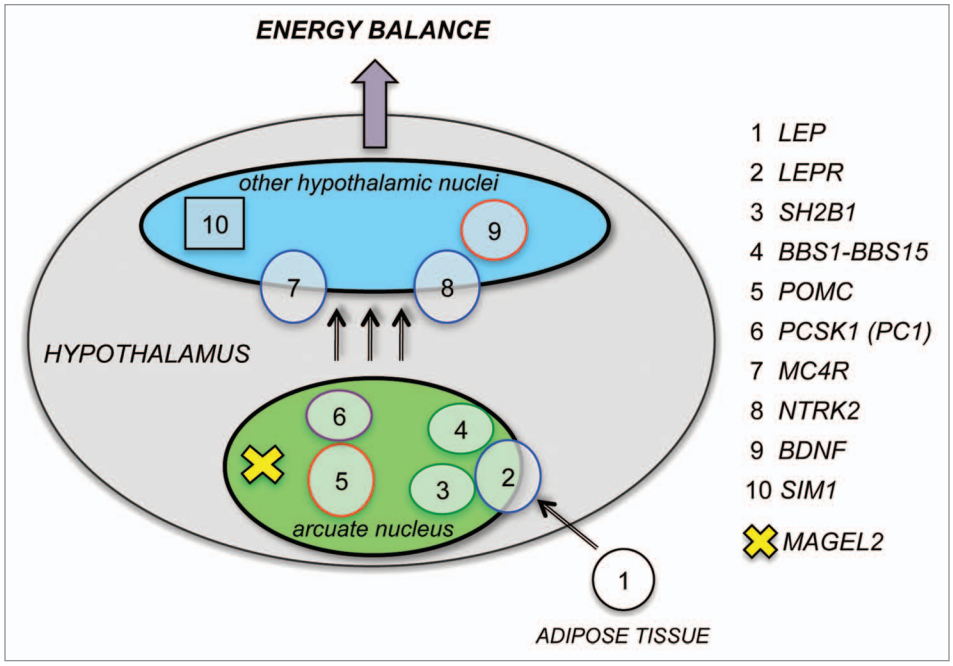

Figure 1. Genes mutated in childhood obesity act in the hypothalamus to regulate energy balance. Mutations that cause rare monogenic or syndromic forms of childhood obesity have been identified in at least 10 different genes, listed on the right. The proteins encoded by these genes act in the hypothalamus (gray) and participate in the leptin - melanocortin pathway that regulates appetite and body weight. *MAGEL2* is one of the genes inactivated in Prader-Willi syndrome, a rare genetic disorder that causes childhood-onset severe obesity. Mercer et al. showed that *Magel2*, the murine ortholog of *MAGEL2*, is essential for the leptin-mediated responses of a specific set of neurons, those that express POMC in the arcuate nucleus of the hypothalamus.

food.[27] Likewise, mice lacking leptin or its receptor are hypoactive, hyperphagic, infertile and obese.[28] Other obese children carry mutations in genes encoding proteins in pathways that intersect leptin circuits, such as those involving the downstream melanocortin or brain-derived neurotrophic factor (BDNF) pathways.[29] For example, variants in the melanocortin-4 receptor (MC4R) gene are the most frequent cause of monogenic obesity, and disruptions to the SIM1 gene cause syndromic obesity by impairing the hypothalamic leptin-melanocortin pathway. Other rare childhood obesity syndromes, such as Bardet-Biedl syndrome (BBS) are caused by mutations in genes encoding components of cilia, which are highly conserved organelles that act as cellular antennae to coordinate signal transduction pathways and control cell growth or differentiation.[30]

Of all the syndromic forms of obesity, Prader-Willi syndrome has been the most perplexing to geneticists and obesity specialists. Like children with leptin pathway mutations, children with PWS have an increased ratio of fat to muscle, relentless hunger that can lead to morbid obesity, as well as reduced voluntary activity and infertility.[24-26] People with PWS have been known to steal, hoard and beg for food, steal money for food, eat spoiled or frozen food and binge on food to the point of gastric distension and sometimes stomach rupture. For this reason, PWS has been described as a genetic model of starvation, because parallel food foraging behavior and reduced energy expenditure are observed in food-deprived animals.[31] In contrast to most human genetic childhood onset obesity disorders where the causative genes disrupt the leptin, melanocortin, BDNF or related pathways, the cause of hyperphagic obesity in PWS in unknown. A priori, a genetic defect involving the leptin - melanocortin pathway is the most parsimonious explanation for obesity in PWS. However, until now, a mechanistic link between any one PWS gene and a specific pathway in the brain had not been made.

PWS is most commonly caused by a microdeletion that occurs on the paternally inherited chromosome 15 and genomic imprinting that silences the maternal allele of six key genes in this region. In this respect, the genetic odds have not been in our favor. Multiple genes, including at least five protein-coding genes and a gene that produces a long non-coding RNA are simultaneously inactivated either by this microdeletion, by uniparental disomy or by a mutation that disrupts the imprinting process. Nature has thrown almost every possible genetic twist at the PWS region: in addition to genomic imprinting, clusters of small nucleolar RNAs are encoded in the introns of the non-coding RNA that also serves as an antisense RNA for the UBE3A gene responsible for Angelman syndrome.[32,33] Other inactivated genes encode proteins of unknown function. One protein-coding gene located in the human PWS region is not found in rodents, complicating the studies of animal models.[34] The PWS region is in the pericentromeric region of chromosome 15, so the genomic DNA is littered with repeated elements and sequence coverage is poor. In spite of this complexity, researchers have been systematically investigating the genes involved, one by

one, in clusters or by studying deletions of the entire region of conserved synteny.[35]

A first clue that *MAGEL2* could be implicated in obesity in PWS came with the observation that *Magel2* is most highly expressed in regions of the hypothalamus that control circadian rhythm and energy balance.[36] Gene targeted knockout mice lacking *Magel2* are overweight and have twice the fat mass, proportionately higher leptin, low lean mass and low bone mass.[16,17] Other hypothalamic deficiencies include blunted circadian rhythm, progressive infertility and neuroendocrine deficits, recapitulating phenotypes seen in people with PWS.[18,20] As described above, excess fat, reduced energy expenditure and infertility are also characteristic of mice with leptin pathway mutations. Mercer et al. therefore tested whether *Magel2* mice have reduced leptin sensitivity and indeed found that, while control mice become anorexic when injected with leptin, adult *Magel2* mice do not consume less food after leptin treatment.

Many neurons in the brain respond to leptin, but the leptin-sensitive neurons considered most critical for energy homeostasis are located in the arcuate nucleus of the hypothalamus.[37] Two distinct neuronal subtypes respond to leptin within the arcuate nucleus: neurons that produce Agouti-related peptide (AgRP) and Neuropeptide Y (NPY) and neurons that produce pro-opiomelanocortin (POMC). Leptin hyperpolarizes (inhibits) AgRP/NPY neurons, reducing the release of orexigenic (appetite-inducing) peptides. In contrast, leptin depolarizes (excites) POMC neurons, promoting the release of anorexigenic (appetite-suppressing) peptides. One such peptide is α-MSH (melanocyte-stimulating hormone), which acts on melanocortin receptors in other brain sites to promote satiety and increase energy expenditure.[38,39] Both the anti-orexigenic and pro-anorexigenic responses to leptin are blunted in animals with acquired (diet-induced obese) or congenital (leptin receptor mutation) leptin insensitivity. To test leptin-mediated electrical activity in hypothalamic neurons, Mercer et al. used whole-cell patch recordings in individual neurons, which surprisingly revealed a defect specific to the anorexigenic POMC neurons in *Magel2* mice. That is, while POMC neurons are present in near normal numbers in the ARC of the hypothalamus, they fail to depolarize in response to leptin, while normal hyperpolarizing responses were detected in AgRP/NPY cells.

The finding that *Magel2* mice have a primary defect in leptin responses in the POMC-expressing class of anorexigenic neurons was intriguing because previous studies had shown that mice with inactivation of the leptin receptor in only the POMC neurons have increased fat mass, but like *Magel2* mice are not massively obese.[40] This is presumably because their AgRP/NPY and other neurons still respond to leptin, as is the case with *Magel2* mice that retain responses to leptin in non-POMC neurons. Likewise, inactivating the signaling protein STAT3 in only POMC neurons causes mild obesity similar to that of *Magel2* mice,[41] and inactivating ciliary function in only POMC neurons is sufficient to cause obesity.[42] The murine Magel2 and human MAGEL2 genes share 72% amino acid similarity,[22,36] and likely share a conserved function in POMC neurons. A defect in the leptin-melanocortin system in children with PWS who lack *MAGEL2* would explain many aspects of their disorder, including but not limited to excess fat mass, lower lean mass, reduced voluntary activity and infertility. Further studies, including clinical trials, will be needed to address whether the melanocortin system can be manipulated pharmacologically to improve appetite control in children with PWS.

We still have far to go in understanding the neural basis for PWS. Mice lacking *Magel2* do not eat voraciously when freely fed, so they do not become morbidly obese like mice with leptin-pathway mutations or like children with PWS whose food environment is not strictly controlled. Their body weight regulation is abnormal though, as they return more slowly to a normal body weight after under- and overfeeding. The loss of other genes, such as the *NDN* gene encoding necdin[43-45] and the clusters of small nucleolar RNAs have also been proposed to account for other endophenotypes in PWS, including neonatal respiratory insufficiency[46] and failure to thrive in infancy.[47] Nonetheless, we now have a foothold into energy imbalance in one of the last remaining and arguably most intriguing rare genetic disorders causing obesity. The new actor on this stage—Magel2—will have to find its niche in the complex protein network in hypothalamic neurons that governs the delicate balance between food intake and energy expenditure. This discovery also adds a new pharmacological target for improving hormone sensitivity and activating pathways downstream of appetitive hormones, both promising avenues for treatment of the obesity that has become rampant in today's society.

Disclosure of Potential Conflicts of Interest

No potential conflict of interest was disclosed.

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