Magel2 knockdown in hypothalamic POMC neurons innervating the medial amygdala reduces susceptibility to diet-induced obesity

Yuna Choi1,2,*, Hyeon-Young Min1,2,*, Jiyeon Hwang1,2, Young-Hwan Jo1,2,3

Hyperphagia and obesity profoundly affect the health of children with Prader–Willi syndrome (PWS). The Magel2 gene among the genes in the Prader–Willi syndrome deletion region is expressed in proopiomelanocortin (POMC) neurons in the arcuate nucleus of the hypothalamus (ARC). Knockout of the Magel2 gene disrupts POMC neuronal circuits and functions. Here, we report that loss of the Magel2 gene exclusively in ARCPOMC neurons innervating the medial amygdala (MeA) causes a reduction in body weight in both male and female mice fed with a high-fat diet. This anti-obesity effect is associated with an increased locomotor activity. There are no significant differences in glucose and insulin tolerance in mice without the Magel2 gene in ARCPOMC neurons innervating the MeA. Plasma estrogen levels are higher in female mutant mice than in controls. Blockade of the G protein–coupled estrogen receptor (GPER), but not estrogen receptor-α (ER-α), reduces locomotor activity in female mutant mice. Hence, our study provides evidence that knockdown of the Magel2 gene in ARCPOMC neurons innervating the MeA reduces susceptibility to diet-induced obesity with increased locomotor activity through activation of central GPER.

DOI 10.26508/lsa.202201502 | Received 25 April 2022 | Revised 12 August 2022 | Accepted 15 August 2022 | Published online 25 August 2022

Introduction

Prader–Willi syndrome (PWS) is a neurogenetic disorder caused by the loss of paternal expression of a cluster of genes in the human 15q11-q13 and mouse 7C regions (Boccaccio et al, 1999; Lee et al, 2000; Resnick et al, 2013). A classic sign of PWS is a constant craving for food, resulting in rapid weight gain in early childhood (Cassidy et al, 2012). Namely, insatiable appetite and hyperphagia are one of the factors that most affect children with PWS. Among the genes in the PWS deletion region, the Magel2 gene appears to be one of the genes responsible for the etiology of PWS. Loss of the mouse Magel2 gene causes abnormalities in growth and fertility and increased adiposity with altered metabolism in adulthood, consistent with some of the pathologies observed in PWS (Bischof et al, 2007; Mercer & Wervick, 2009). Although the exact cellular mechanisms underlying the abnormalities remain determined, a recent study shows deficits in secretory granule abundance and neuropeptide production in the hypothalamus of Magel2-null mice (Chen et al, 2020). For instance, Magel2-null mice have lower levels of α-melanocyte–stimulating hormone (α-MSH) derived from proopiomelanocortin (POMC) (Chen et al, 2020). As the arcuate nucleus of the hypothalamus (ARC) highly expresses POMC, the loss of Magel2 lowers POMC mRNA (Kozlov et al, 2007; Maillard et al, 2016; Chen et al, 2020), a loss of function of the Magel2 gene may disrupt the central melanocortin system, causing impairments in whole-body energy metabolisms.

POMC neurons in the ARC play a significant role in regulating food intake and body weight (Plum et al, 2006; Bumaschny et al, 2012; Greenman et al, 2013; Lam et al, 2015; Yeo et al, 2021). Acute stimulation of a subset of ARCPOMC neurons reduces feeding (Jeong et al, 2018; Wei et al, 2018). For instance, activation of temperature-sensitive transient receptor potential cation channel subfamily V member 1 (TRPV1)–expressing POMC neurons decreases feeding (Jeong et al, 2018) and optogenetic stimulation of mature ARCPOMC neurons in POMCERT mice also lowers food intake (Wei et al, 2018). In contrast, ablation of ARCPOMC neurons in adult mice results in hyperphagia and obesity (Zhan et al, 2013). It has been shown that Magel2-null mice exhibit impaired POMC neural circuits and functions (Mercer et al, 2013; Maillard et al, 2016; Oncul et al, 2018). There are fewer ARCPOMC neurons in Magel2-null mice than in controls (Mercer et al, 2013). ARCPOMC axonal projections to the paraventricular (PVN) and dorsomedial (DMH) hypothalamus are significantly reduced by Magel2 deletion (Maillard et al, 2016). Furthermore, the basal spontaneous activity of ARCPOMC neurons is lower in Magel2-null mice than in controls (Oncul et al, 2018). Hence, disrupted ARCPOMC neural circuits may result in changes in metabolic homeostasis in Magel2-null mice.
Among the downstream targets of ARCPOMC axonal projections, POMC projections to the PVN and medial amygdala (MeA) are implicated in controlling feeding behavior through activation of the melanocortin receptor type 4 (MC4R) (Balthasar et al, 2005; Liu et al, 2013; Shah et al, 2014; Kwon & Jo, 2020). We recently demonstrated that optogenetic stimulation of the ARCPOMC→MeA pathway decreased liquid food intake blocked by the MC4R antagonist (Kwon & Jo, 2020). Given that the ARCPOMC→MeA pathway regulates feeding, we specifically examined the role of the Magel2 gene in ARCPOMC neurons innervating the MeA in controlling food intake and body weight gain. Unexpectedly, knockdown of the Magel2 gene in MeA-projecting ARCPOMC neurons reduced rather than increased body weight without changing food intake in mice when fed a high-fat diet (HFD). Importantly, this anti-obesity effect was associated with increased locomotor activity in male and female mice. Our results provide cellular evidence that MAGEL2 in MeA-projecting ARCPOMC neurons plays an essential role in controlling energy balance.

Results

**ARCPOMC neurons express MAGEL2**

High expression of Magel2 mRNAs was detected in the ARC (Kozlov et al, 2007; Maillard et al, 2016; Chen et al, 2020), but the neurochemical identity of Magel2-expressing neurons in the area remains unknown. We first examined if ARCPOMC neurons express MAGEL2. We used POMCCre:Rosa26-GFP mice, in which the POMCCRE transgene causes cell-specific recombination to induce expression of eGFP from the Rosa26 promoter and an antibody directed against MAGEL2 (Chen et al, 2020). MAGEL2 expression was detected in the ARC as described previously (Chen et al, 2020). We found that more than 60% of ARCPOMC neurons in male POMCcre:Rosa26-GFP mice were positive for MAGEL2 (n = 1,771 of 2,756 POMC neurons; Fig 1A and B). Similarly, most of the ARCPOMC neurons were labeled with the anti-MAGEL2 antibody in female POMCcre:Rosa26-GFP mice (n = 3,265 of 4,543 neurons; Fig 1C and D). Our immunostaining results support the interpretation that MAGEL2 may play a role in controlling ARCPOMC neuron functions.

**Knockdown of the Magel2 gene exclusively in MeA-innervating ARCPOMC neurons causes a reduction in body weight in male mice fed with a high-fat diet**

PWS animal models, such as Magel2- and Snord116-null mice fed with a standard chow diet did not develop the delayed-onset obesity described in PWS (Bischof et al, 2007; Qi et al, 2016). Interestingly, although the overall body weight in Magel2-null mice was not different from that in the control group, Magel2-null mice exhibited a significant increase in adiposity (Bischof et al, 2007). We thus sought to determine if the nutrient excess induces diet-induced obesity in our animal model. To investigate the role of the Magel2 gene in ARCPOMC neurons innervating the MeA in controlling energy balance, we knocked down the Magel2 gene exclusively in ARCPOMC neurons that project to the MeA with the use of CRISPR-Cas9 technology as described in our prior study (Jeong et al, 2018). We crossed the POMCcre strain with the floxed-stop Cas9-eGFP strain to generate POMCcre:Cas9-eGFP mice. We bilaterally
injected a retrograde adeno-associated virus (AAV) encoding mouse *Magel2* single guide RNA (retroAAV-Magel2 sgRNA) into the MeA of POMCre:Cas9-eGFP and POMCre mice (Figs 2A and S1). After viral injection, mice were given a high-fat diet for 10 wk. To validate our experimental approach, the *Magel2* gene expression in the ARC at 10 wk post-viral injections was analyzed by reverse transcription-quantitative polymerase chain reaction (RT-qPCR). We found that male POMCre:Cas9-eGFP mice receiving retroAAV-Magel2 sgRNA exhibited lower *Magel2* expression in the ARC compared with control mice (Fig 2A). In addition to reduced *Magel2* gene expression, the number of MAGEL2-positive cells in the ARC decreased in POMCre:Cas9-eGFP mice receiving retroAAV-Magel2 sgRNA injection (Fig 2B), demonstrating the feasibility and efficacy of retroAAV-Magel2 sgRNA.

When fed HFD for 10 wk, we found that male POMCre:Cas9-eGFP mice receiving retroAAV-Magel2 sgRNA significantly gained less body weight than controls (Fig 2C). Lower body weight was associated with a reduction in body fat mass but not lean mass (Fig 2D and E). We next asked if this body weight loss is attributed to either reduced energy intake, increased energy expenditure, or both. Although body weight was significantly different during high-fat feeding, daytime and nighttime food consumption between the experimental and control groups was not significantly different (Fig 2F).

The absence of reduced food intake suggests that changes in energy expenditure may result in weight loss. We investigated if there is enhanced energy expenditure resulting from either enhanced basal metabolic rate and/or physical activity. We placed mice in metabolic cages to measure energy expenditure (measured as O₂ consumption [VO₂] and respiratory exchange ratio [RER]). There was no significant difference in O₂ consumption and RER between the two groups (Fig 2G and H). Interestingly, nighttime locomotor activity in male POMCre:Cas9-eGFP mice receiving retroAAV-Magel2 sgRNA was higher than that in the control group (Fig 2I and J). Unexpectedly, total energy expenditure between the groups was similar (Fig 2K-M). Hence, our results revealed that the loss of function of the *Magel2* gene in ARC converts neurons innervating the MeA resulted in increased physical activity.

Given that POMCre:Cas9-eGFP mice receiving retroAAV-Magel2 sgRNA injection exhibited lowered body weight gain, we sought to determine if knockdown of the *Magel2* gene improves glucose homeostasis. We first quantified basal (non-fasting) and fasting glucose levels. There were no significant differences in basal and fasting blood glucose levels between the groups (Fig 3A and B). And then, we performed glucose tolerance tests to assess the ability of male POMCre:Cas9-eGFP mice receiving retroAAV-Magel2 sgRNA injection to dispose of a glucose load. *Magel2* knockdown did not change glucose clearance (Fig 3C). We also carried out insulin tolerance tests to assess glucose levels over time to an i.p. insulin injection and found that insulin tolerance was not affected by *Magel2* knockdown (Fig 3D). Hence, lower body weight in male POMCre:Cas9-eGFP mice receiving retroAAV-Magel2 sgRNA injection did not occur in conjunction with altered glucose homeostasis. We analyzed plasma levels of leptin and insulin that play a key role in controlling satiety, insulin sensitivity, and glucose homeostasis. *Magel2* knockdown had no effects on plasma leptin and insulin levels (Fig 3E and F).

**Knockdown of the *Magel2* gene exclusively in MeA-innervating ARC POMC neurons causes a reduction in body weight in female mice fed with a high-fat diet**

As *Magel2* knockdown in ARC POMC neurons innervating the MeA lowered body weight gain in male mice, we examined if female mice without the *Magel2* gene in ARC POMC neurons innervating the MeA similarly do not develop DIO. Injection of retroAAV-Magel2 sgRNA into the MeA of female POMCre:Cas9-eGFP mice significantly decreased expression of the *Magel2* gene and protein in the ARC (Fig 4A and B). Likewise, female mice receiving retroAAV-Magel2 sgRNA injection gained less body weight than controls during high-fat feeding (Fig 4C). Fat mass in mutant female mice significantly differed from that in controls, whereas lean body mass was similar between the experimental groups (Fig 4D and E).

We also found that knockdown of the *Magel2* gene in female mice did not change food consumption (Fig 4F). Both groups consumed a similar amount of HFD during the day and dark phases. In addition, measurement of O₂ consumption revealed that female POMCre:Cas9-eGFP mice receiving retroAAV-Magel2 sgRNA also exhibited no significant difference in VO₂ compared with the control group, although there was a trend toward an increase in VO₂ in the mutant mice (Fig 4G). The RER was not significantly different between the experimental groups (Fig 4H). Interestingly, similarly to males, total locomotor activity in the dark phase was significantly higher in female POMCre:Cas9-eGFP mice receiving retroAAV-Magel2 sgRNA compared with that in the control group (Fig 4I and J). In addition, female mice without the *Magel2* gene in ARC POMC neurons innervating the MeA exhibited higher daytime locomotor activity compared with the controls (Fig 4I and J). A trend toward an increase in TEE was observed in female mice receiving retroAAV-Magel2 sgRNA injection (Fig 4K-M). Hence, the loss of function of the *Magel2* gene in ARC POMC neurons caused increased physical activity in female mice as well.

We also sought to determine if knockdown of the *Magel2* gene improves glucose homeostasis. The experimental and control groups exhibited no significant differences in basal and fasting blood glucose levels (Fig 5A and B). We found no alterations in glucose and insulin tolerance (Fig 5C and D). No significant differences were found in plasma insulin levels between the two groups, but the mutant mice showed a trend of lower levels of insulin (Fig 5E and F). Collectively, our results suggest that increased locomotor activity contribute to body weight loss after *Magel2* knockdown in ARC POMC neurons innervating the MeA in female mice as well.

**Knockdown of the *Magel2* gene in MeA-projecting ARC POMC neurons elevates plasma estrogen levels in female mice**

Estrogen played a critical role in driving physical activity in female mice through activation of the nuclear estrogen receptor-α (ER-α) in the brain, including the hypothalamus and MeA (Ogawa et al, 2003; Xu et al, 2011, 2015; Krause et al, 2021). In addition, ER-α KO mice exhibited increased adiposity, insulin resistance, and impaired glucose tolerance in males and females (Heine et al, 2000). Central ER-α KO mice also displayed hyperphagia, increased body weight, adiposity, and reduced physical activity (Xu et al, 2011). Thus,
Figure 2. Loss of the Magel2 gene in ARC^{POMC} neurons innervating the MeA causes a reduction in body weight while increasing locomotor activity in male mice fed with HFD.

(A) Schematic diagram of the experimental configuration. Retrograde AAV-Magel2 sgRNA viruses were bilaterally injected into the MeA of POMC^{Cre} and POMC^{Cre:Cas9-GFP} mice. Left panel: Summary plot showing relative expression of the Magel2 gene in the ARC of POMC^{Cre} (open circle; n = 10 mice) and POMC^{Cre:Cas9-GFP} (closed circle; n = 11 mice) mice receiving retrograde AAV-Magel2 sgRNA viral injection to the MeA. There was a significant difference in Magel2 expression between the two groups. Two-tailed t test, ***P < 0.001. (B) Images of confocal fluorescence microscopy showing MAGEL2 expression in the ARC of POMC^{Cre} (left) and POMC^{Cre:Cas9-GFP} (right) mice receiving retrograde AAV-Magel2 sgRNA viral injection to the MeA. Scale bar, 30 μm. Right panel: Summary plot showing the number of MAGEL2-positive cells in the ARC of POMC^{Cre} (left; n = 4 mice) and POMC^{Cre:Cas9-GFP} (right; n = 4 mice) mice receiving retrograde AAV-Magel2 sgRNA viral injection. (C) Pooled data of body weight obtained from POMC^{Cre} (open circle; n = 17 mice) and POMC^{Cre:Cas9-GFP} (closed circle; n = 14 mice) mice receiving retrograde AAV-Magel2 sgRNA viral injection to the MeA. The loss of the Magel2 gene in ARC^{POMC} neurons innervating the MeA resulted in body weight loss in male mice fed with HFD for 10 wk. Two-way repeated measures ANOVA followed by Sidak multiple comparisons test (between the groups, F(1, 16) = 8.1, *P < 0.05). (D, E) Pooled data of body composition from POMC^{Cre} (n = 13 mice) and POMC^{Cre:Cas9-GFP} (n = 12 mice)
we hypothesized that higher locomotor activity and altered energy expenditure in POMC CRECass-GFP mice receiving retroAAV-Magel2 sgRNA may be due to enhanced estrogen/ER-α signaling in the brain. We thus examined if Magel2 knockdown in ARC POMC neurons innervating the MeA elevates plasma estrogen levels. We found that plasma estrogen levels were significantly higher in female POMC CRECass-GFP mice receiving retroAAV-Magel2 sgRNA injection than in the control group (Fig 6A). Although we failed to detect plasma estrogen in male mice because their estrogen levels were outside the detection range, levels of plasma testosterone in male POMC CRECass-GFP mice receiving retroAAV-Magel2 sgRNA mice did not differ from those in the control group (POMC CRE+Magel2 sgRNA, 294 ± 36 pg/ml, n = 10 mice; POMC CRECass-GFP +Magel2 sgRNA, 312 ± 36 pg/ml, n = 11 mice, P > 0.05).

Hence, we sought to determine if blockade of central ER-α could reverse the effects of Magel2 knockdown on locomotor activity. We infused the ER-α antagonist ICI 182,780 (Fulvestrant; 40 ng/day) into the lateral ventricle via osmotic pumps and placed mice in metabolic cages. We chose the dose based on previous studies, in which dose of the ER-α antagonist completely blocked the effect of central ER-α (Xue et al, 2007, 2008; Stell et al, 2008). Infusion of the ER-α antagonist did not change VO2 and RER (Fig 6B and C). In contrast to our expectation, female POMC CRECass-GFP mice receiving retroAAV-Magel2 sgRNA injection responded to ICI 182,780 with a significant increase rather than decrease in physical activity in the dark phase (Fig 6D and E). There was no significant difference in total energy expenditure between the groups (Fig 6F–H). These unexpected findings suggest that activation of the nuclear ER-α may not increase physical activity in female POMC CRECass-GFP mice receiving retroAAV-Magel2 sgRNA injection.

Central estrogen-GPER interaction causes increased locomotor activity in female mice

In addition to the nuclear ER-α which predominantly regulates transcription (Fuentes & Silveira, 2019), the G-protein-coupled estrogen receptor (GPER; previously known as GPR30) was found in the brain and played a role in metabolic regulation (Prossnitz & Barton, 2011). Although the ER-α antagonist ICI 182,780 has been widely used to treat breast cancer in postmenopausal women (Nathan & Schmid, 2017), it is described that this substance can also activate GPER (Filardo et al, 2000; Meyer et al, 2010). It is thus possible that activation of central GPER may promote physical activity in our preparations. We first carried out immunostaining with an anti-GPER antibody to investigate if GPER is expressed in the VMH and MeA as these structures are implicated in estrogen-induced physical activity in mice (Xu et al, 2015; Krause et al, 2021). Immunostaining revealed that GPER was detected in the VMH as well as the MeA (Fig 7A), suggesting that increased estrogen levels may elevate locomotor activity via activation of central GPER.

Next, we sought to determine if blockade of central GPER could reduce increased locomotor activity observed in female mice receiving retroAAV-Magel2 sgRNA injection to the MeA. The potent GPER antagonist G15 was infused into the lateral ventricle via osmotic pumps. Like ICI 182,780, the GPER antagonist did not alter VO2 and RER (Fig 7B and C). However, in contrast to the effects of ICI 182,780 on physical activity, we found that female POMC CRECass-GFP mice receiving retroAAV-Magel2 sgRNA injection did not exhibit increased locomotor activity (Fig 7D and E). There was no significant difference in TEE (Fig 7F–H). Hence, our results suggest that GPER expressed in the VMN and MeA may contribute to the control of locomotor activity in female POMC CRECass-GFP mice receiving retroAAV-Magel2 sgRNA injection.

Discussion

Our current study provided physiological evidence for the role of the Magel2 gene in ARC POMC neurons innervating the MeA in the control of energy balance. Prior studies demonstrated Magel2 mRNA expression in the hypothalamus, particularly the ARC (Kozlov et al, 2007; Maillard et al, 2016; Chen et al, 2020). We found that most of the ARC POMC neurons expressed MAGEL2. The knockdown of the Magel2 gene exclusively in ARC POMC neurons innervating the MeA protected against DIO in both male and female mice, consistent with prior studies with PWS mouse models, including mice lacking the Magel2 and Snord116 genes (Bischof et al, 2007; Qi et al, 2016). The cellular mechanisms underlying the anti-obesity effects appear to be sex-dependent. Both male and female mutant mice exhibited higher locomotor activity than the control groups. Interestingly, it seems likely that sex hormone levels played a key role in regulating locomotor activity in females. In fact, there was a significant increase in estrogen levels in female POMC CRECass-GFP mice receiving retroAAV-Magel2 sgRNA injection. In contrast, no difference in testosterone levels was found between the male groups. Finally, blockade of GPER but not ER-α completely abolished the effect of loss of the Magel2 gene in ARC POMC neurons innervating the MeA. This was observed in female mice only. Hence, central estrogen and GPER interaction played a critical role in controlling energy balance in our PWS mouse model.

Loss of imprinted genes at the PWS-chromosome domain, such as the Magel2, Snord116, and Ndn genes in mice, caused behavioral and neuroendocrine alterations reminiscent of PWS (Muscatelli et al, 2000; Bischof et al, 2007; Kozlov et al, 2007; Qi et al, 2016; Burnett et al, 2017). For instance, mice lacking the Ndn gene exhibited early post-natal lethality partly because of a respiratory defect (Gerard et al, 1999; Muscatelli et al, 2000). This finding correlated with neonatal respiratory distress observed in PWS patients (Alfaro et al, 2019). In addition, these mutant mice had fewer oxytocin neurons in the paraventricular nucleus of the hypothalamus than controls.
et al., 2017). In addition, mice de
their weight loss, the
adulthood (Ding et al., 2008; Qi et al., 2016; Burnett et al., 2017). Despite
with a standard chow diet caused growth retardation, such as
the germline and paternal deletion of the
imprinted genes causes the development of obesity, as ob-
(PWS) imprinted genes causes the development of obesity, as ob-
the hypothalamus, pancreatic islet, and stomach (Burnett et al., 2017). A down-regulation of P1C reduced circulating levels of
insulin, ghrelin, and growth hormone-releasing hormone (Burnett et al., 2017). Consequently, deficiencies in prohormone processing may result in the neuroendocrine phenotype of PWS, including hypogonadism, short stature, and type 2 diabetes (Burnett et al., 2017). Similarly, loss of the Magel2 gene in mice caused decreased neuropeptide production in the hypothalamus because of down-regulation of neuropeptide processing enzymes, including PC1, PC2, and carboxypeptidase E (Chen et al., 2020). Consequently, hypothalamic and plasma levels of neuropeptides such as vasopressin, oxytocin, somatostatin, and agouti-related peptide were lower in
Magel2-null mice than in controls (Chen et al., 2020). Interestingly, levels of α-MSH produced from the POMC precursor by POMC posttranslational modification enzymes, including PC1, PC2, and CPE were also significantly lower in Magel2 KO mice than in controls (Chen et al., 2020). As hypothalamic α-MSH and its cognate receptors play a major role in regulating energy balance and glucose ho-
meostasis (Wallingford et al., 2009; Shah et al., 2014; Schneeberger et al., 2015; Tooke et al., 2019), a disruption in the central melanocortin system in PWS may cause behavioral and neuroendocrine changes.

Our present study revealed that most of the ArcPOMC neurons expressed MAGEL2, supporting the previous findings that the loss of the Magel2 gene disrupted hypothalamic POMC neural circuits and functions (Merce
et al., 2013; Maillard et al., 2016; Oncu et al., 2018). For instance, Magel2-null mice exhibited altered ArcPOMC neuron electric activity (Oncu et al., 2018), impaired ArcPOMC neuronal projections to the PVH, ARC, and DMH (Maillard et al., 2016), and lack of leptin’s anorexic effect (Merce
et al., 2013). Prior studies (Elias et al., 1998, 1999; King & Hentges, 2011; Dicken et al., 2012; Henry et al., 2015; Koch et al., 2015; Wang et al., 2015; Campbell et al., 2017; Chen et al., 2017; Lam et al., 2017; Biglari et al., 2021), including our own work (Lee et al., 2015; Jeong et al., 2016; Kwon & Jo, 2020) demonstrated that ArcPOMC neurons were neurochemically and neuroanatomically heterogeneous. Our recent studies further showed functional heterogeneity of ArcPOMC neurons (Jeong et al., 2018; Kwon et al., 2020; Kwon & Jo, 2020). In other words, neurochemically and neuroanatomically distinct subpopulations of POMC neurons had distinct target sites and metabolic functions.

In this study, we knocked down the expression of the Magel2 gene exclusively in the ArcPOMC→MeA projection because ArcPOMC projections to the MeA controlled acute food intake through

![Figure 3. Loss of the Magel2 gene in ArcPOMC neurons innervating the MeA](image)
Figure 4. Loss of the Magel2 gene in ARC\textsuperscript{POMC} neurons innervating the MeA causes a reduction in body weight while increasing locomotor activity in female mice fed with HFD.

(A) Summary plot showing relative expression of the Magel2 gene in the ARC of POMC\textsuperscript{Cre} (open circle; n = 13 mice) and POMC\textsuperscript{CreCas9-GFP} (closed circle; n = 11 mice) mice receiving retrograde AAV-Magel2 sgRNA viral injection to the MeA. Two-tailed t test, ***P < 0.001. (B) Images of confocal fluorescence microscopy showing MAGEL2 expression in the ARC of mice with (left) and without (right) the Magel2 gene in ARCPOMC neurons innervating the MeA. Scale bar, 30 μm. Right panel: Summary plot showing the number of MAGEL2-positive cells in the ARC. *P < 0.05. (C) Pooled data of body weight obtained from POMC\textsuperscript{Cre} (open circle, n = 24 mice) and POMC\textsuperscript{CreCas9-GFP} (closed circle, n = 18 mice) mice receiving retrograde AAV-Magel2 sgRNA viral injection to the MeA. A significant difference in body weight was observed between the groups. Two-way repeated measures ANOVA followed by Sidak multiple comparisons test (between the groups, F(1, 40) = 8.39, **P < 0.01). (D, E) Pooled data of body composition from POMC\textsuperscript{Cre} (n = 13 mice) and POMC\textsuperscript{CreCas9-GFP} (n = 12 mice) mice receiving retrograde AAV-Magel2 sgRNA viral injection to the MeA. Two-tailed t test, *P < 0.05. (F) Pooled data showing no significant difference in food intake between the groups (POMC\textsuperscript{Cre}, n = 8 mice; POMC\textsuperscript{CreCas9-GFP}, n = 6 mice). (G, H) Summary plot showing VO\textsubscript{2} and respiratory exchange ratio (H) between the groups. No significant difference in VO\textsubscript{2} and respiratory exchange ratio was observed in mice without the Magel2 gene in ARCPOMC neurons innervating the MeA (POMC\textsuperscript{Cre}, n = 5 mice; POMC\textsuperscript{CreCas9-GFP}, n = 6 mice). (I) Pooled data showing increased total and ambulatory activity in mice without Magel2 knockdown in hypothalamic POMC neurons. Choi et al. https://doi.org/10.26508/lsa.202201502 vol 5 | no 11 | e202201502 7 of 14
activation of MC4R (Balthasar et al., 2005; Liu et al., 2013; Kwon & Jo, 2020). We expected that a loss of the Magel2 gene in this subset of ARC\(^{POMC}\) neurons would disrupt this melanocortin pathway. As a result, the HFD challenge would result in DIO in POMC\(^{Cre-Cas9-GFP}\) mice receiving retroAAV-Magel2 sgRNA injection to the MeA. Contrary to our expectation, knockdown of the Magel2 gene in MeA-\(\alpha\)-innervating ARC\(^{POMC}\) neurons protected the mutant mice from DIO, consistent with the prior studies with Snord116 KO and Magel2 KO male animals (Bischof et al., 2007; Qi et al., 2016). We found no difference in food intake relative to lean mass between the male mutant and control groups, meaning that they did not develop hyperphagia. However, reduced body weight was associated with a decreased percentage of fat mass, consistent with the finding in Snord116 KO mice (Qi et al., 2016). Given the reported similarity of our male mutant mice to Snord116-deficient mice (e.g., reduced body, no effect on food intake, decreased percentage of fat mass, increased locomotor activity at the dark phase) (Ding et al., 2008; Qi et al., 2016), it appears that both genes do not contribute to the control of energy intake. As both mutant mice exhibited increased locomotor activity, this may reduce susceptibility to DIO.

The difference in body weight between the two groups during high-fat feeding would be due partly to altered energy expenditure. Indirect calorimetry revealed no significant differences in daytime and nighttime O\(_2\) consumption. Although we failed to detect a significant difference in energy expenditure between the two groups, it does not mean that there was no difference in energy expenditure between the groups during high-fat feeding. It is highly possible that our system may not be able to detect a subtle change in energy expenditure because of the beginning of high-fat feeding may prevent weight gain in our animal model. In fact, female mice lacking the Magel2 in ARC\(^{POMC}\) neurons innervating the MeA exhibited an trend toward an increase in total energy expenditure. In addition, POMC\(^{Cre-Cas9-GFP}\) male mice receiving retroAAV-Magel2 sgRNA injection to the MeA exhibited higher locomotor activity in the dark phase compared with the control group. Locomotor activity in Snord116-null male mice significantly increased only in the dark phase as well (Qi et al., 2016). Thus, it is plausible that increased physical activity in male mice without imprinted genes at the PWS-chromosome domain would cause body weight loss. Although male POMC\(^{Cre-Cas9-GFP}\) mice receiving retroAAV-Magel2 sgRNA injection to the MeA were resistant to DIO, there were no improvements in glucose homeostasis and insulin sensitivity in our preparations. Basal and fasting glucose levels in the mutant mice did not differ from those in their controls, and no difference in insulin tolerance was detected between the groups. In addition, basal insulin levels and insulin tolerance were not altered by loss of the Magel2 gene in MeA-\(\alpha\)-innervating ARC\(^{POMC}\) neurons.

Resistance to DIO appeared partly because of increased locomotor activity in the dark phase. What causes an increase in physical activity in PWS animal models? Prior studies demonstrated that central ER\(\alpha\) could control physical activity in female mice (Ogawa et al., 2003; Xu et al., 2011, 2015; Krause et al., 2021). For example, mice lacking central ER\(\alpha\) exhibited reduced physical activity, resulting in increased body weight (Xu et al., 2011). Activation of ER\(\alpha\) in the VMH promoted physical activity in female mice via increased expression of Mc4r transcripts in the VMH (Krause et al., 2021). Moreover, chemogenetic stimulation of VMH\(\alpha\)-ME\(\alpha\) neurons increased spontaneous physical activity in both male and female mice which was sufficient to reduce body weight (Krause et al., 2021). Selective deletion of ER\(\alpha\) in single-minded (SIM1) neurons in the MeA also showed a significant reduction in physical activity in male mice (Xu et al., 2015). In our preparations, increased physical activity was closely associated with elevated estrogen levels in POMC\(^{Cre-Cas9-GFP}\) female mice receiving retroAAV-Magel2 sgRNA injection to the MeA. Moreover, we recently showed that most of the ER\(\alpha\)-positive cells in the MeA also expressed MC4Rs (Kwon & Jo, 2020). It is plausible that estrogen would regulate locomotor activity via activation of ER\(\alpha\) and MC4R-co-expressing neurons in the MeA. However, central infusion of the broad ER\(\alpha\)-antagonist ICI 182,780 failed to block the effect of the loss of Magel2 in ARC\(^{POMC}\) neurons innervating the MeA on locomotor activity. Instead, ICI 182,780 infusion increased locomotor activity in female POMC\(^{Cre-Cas9-GFP}\) mice receiving retroAAV-Magel2 sgRNA injection to the MeA.

Although this ER\(\alpha\)-antagonist has been widely used to treat breast cancer in postmenopausal women, this drug is known to activate GPER. Increased locomotor activity only in POMC\(^{Cre-Cas9-GFP}\) female mice receiving retroAAV-Magel2 sgRNA injection to the MeA might be due to activation of GPER in the brain. Indeed, treatment with the GPER antagonist effectively blocked the effect of the loss of the Magel2 gene in POMC neurons on locomotor activity in female mice. GPER was found in the hypothalamus, including the VMH and the MeA in both male and female rodents (Hazell et al., 2009; Marraudino et al., 2021). As ER\(\alpha\) in these brain structures promoted physical activity in mice (Ogawa et al., 2003; Xu et al., 2011, 2015; Krause et al., 2021), ER\(\alpha\) and GPER may coordinately or independently regulate locomotor activity and eventually, energy balance. In fact, treatment with the GPER agonist caused a reduction of body weight in ovariectomized female mice and prevented body weight gain in male DIO mice (Sharma et al., 2020). Although plasma estrogen levels in male mice were outside of the detection range in our preparations, it is possible that GPER may play a role in controlling locomotor activity and energy balance in male mice as well. In fact, aromatase that converts testosterone into estrogen was highly expressed in the MeA and the number of aromatase-positive cells in the MeA was higher in males than in females (Wu et al., 2009). In addition, GPER was expressed in the MeA in both males and females (Llorente et al., 2020). Thus, a local increase in estrogen levels in the MeA may activate GPER in male mice as well. However, we could rule out the possibility that estrogen-independent mechanisms may contribute to the control of body weight and locomotor activity in our animal model. Collectively, deletion of the Magel2 gene in the ARC\(^{POMC}\)→MeA neural circuit elevated estrogen levels, which may result in activation of central GPER in female mice. This would promote physical activity, causing body weight...
Figure 5. Loss of the Magel2 gene in ARCPOMC neurons innervating the MeA does not change glucose homeostasis in female mice fed with HFD. (A, B) Summary plots showing non-fasting and fasting blood glucose levels in POMC Cre (open circle) and POMC Cre:Cas9+GFP (closed circle) mice receiving retrograde AAV-Magel2 sgRNA viral injection to the MeA (POMC Cre, n = 19 mice and POMC Cre:Cas9+GFP, n = 8 mice for non-fasting blood glucose; POMC Cre, n = 17 mice and POMC Cre:Cas9+GFP, n = 9 mice for fasting blood glucose). (C) Pooled data showing GTT in mice with and without the Magel2 gene in ARCPOMC neurons innervating the MeA (left). Right panel: graphs showing AUC values obtained from GTT experiments (two-tailed t test, POMC Cre, n = 6 mice; POMC Cre:Cas9+GFP, n = 6 mice). (D) Pooled data showing ITT in mice with and without the Magel2 gene in ARCPOMC neurons innervating the MeA. No significant difference was observed between the groups. (E, F) Summary plots showing levels of plasma leptin and insulin in mice with and without the Magel2 gene in ARCPOMC neurons innervating the MeA (POMC Cre, n = 5 mice; POMC Cre:Cas9+GFP, n = 7 mice for leptin; POMC Cre, n = 5 mice, POMC Cre:Cas9+GFP, n = 6 mice for insulin).

Materials and Methods
Ethics statement
All mouse care and experimental procedures were approved by the Institutional Animal Care Research Advisory Committee of the Albert Einstein College of Medicine and were performed in accordance with the guidelines described in the NIH guide for the care and use of laboratory animals. Stereotaxic surgery and viral injections were performed under isoflurane anesthesia.

Animals
Mice used in this study included POMC-Cre (stock # 005965), floxed-stop Cas9-eGFP (stock # 026175), and floxed-stop Rosa26-eGFP mice (stock # 004077) that we purchased from the Jackson Laboratory. Both female and male mice of mixed C57BL/6J, FVB, and 129 strain backgrounds were used. Animals were housed in groups in cages under conditions of controlled temperature (22°C) with a 12:12 h light–dark cycle and fed a standard chow diet with ad libitum access to water. After stereotaxic surgery, mice were given a high-fat diet (20% calories by carbohydrate, 20% by protein, and 60% by fat, 5.21 kcal/g, D12492; Research Diet) for 10 wk.

Stereotaxic surgery and viral injections
To knock down the Magel2 gene in ARCPOMC neurons innervating the MeA, retrograde AAV-PGK-loxp-tdTomato-loxp-U6-mouse Magel2 sgRNA viruses (titer, 1 × 1013 pfu/ml) were generated at Applied Biological Materials Inc (ABM). The sgRNAs were designed to target to the consensus coding sequence (CCDS) 52264.1 region of mouse Magel2 (NM_013779.2). The sequences of Magel2 sgRNA were the following: (1) sgRNA1: cgccagctaagtacgaatctg, (2) sgRNA2: gtagggcgctcctagaatactg, and (3) sgRNA3: atgccgtagctgcgcagtagctgc. 6- and 7-wk-old mice (males, ~20 g and females, ~18 g) were anesthetized deeply with 3% isoflurane and placed in a stereotaxic apparatus (David Kopf Instruments). A deep level of anesthesia was maintained throughout the surgical procedure. Under isoflurane anesthesia (2%), retrograde AAV-PGK-loxp-tdTomato-loxp-U6-mouse Magel2 sgRNA viruses (200 nl/per site, titer, 1 × 1013 pfu/ml) were bilaterally injected into the MeA of POMC Cre and POMC Cre:Cas9+GFP (AP, −1.58 mm; ML, ± 2 mm; DV, −5 mm). A 2.5 μl Hamilton syringe, having a 33-G needle was used to inject a volume of 50 nl viruses every 5 min. The Hamilton syringe tip was left in place for 10 min after delivering viruses to prevent backflow of viral solution up the needle track.

Measurement of food intake, body weight, body composition, and blood glucose levels
Mice were individually housed for a week for acclimation, and daily food intake was measured accurately manually by an investigator (regular weighing of food, control of spillage, and calculation of disappearance of grams) over 5 d at 10 wk post viral injection. Body weight was measured weekly at 9 AM. Body composition for fat mass and fat-free mass was assessed by ECHO MRI at our animal physiology core.

Blood samples were collected from the mouse tail, and a small drop of blood was placed on the test strip of a glucose meter. Non-fasting basal glucose levels were measured at 9:00 AM. Fasting blood glucose levels were measured after an overnight fast once at 10 wk.

Assessment of glucose tolerance and insulin tolerance
For GTT, experimental and control mice at 10 wk post viral inoculation were fasted for 18 h (5:00 PM–11:00 AM). A sterile glucose solution was i.p. administered at a concentration of 2 g/kg (glucose/body weight) at time 0. The blood glucose levels were
measured at 15, 30, 60, 90, and 120 min after glucose injection. Blood glucose levels versus time after glucose injection were plotted, and the area under the curve was calculated and compared between the experimental and control groups.

For ITT, mice were fasted for 5 h (9:00 AM–2 PM). Blood glucose levels were measured at 0, 15, 30, 60, 90, and 120 min after i.p. injection of insulin (1 U/kg). We immediately injected glucose (2 g/kg) if the mice appeared ill because of insulin-induced hypoglycemia.

ICV drug infusion

Mice were maintained under isoflurane anesthesia and placed in a stereotaxic apparatus. Under aseptic conditions, sterile guide cannulas were stereotaxically implanted into the lateral ventricle (AP, −0.22 mm; ML, +1 mm; DV −2.5 mm) for infusion of ICI 182,780 (40 ng/day, sc-203435A; Santa Cruz Biotechnology) or G15 (5 μg/day, 14673; Cayman). The guide cannulas were connected to osmotic pumps (1002W; RWD Life Science), and drugs were infused at a rate of 0.25 μl/hour for 10 d.

Assessment of energy expenditure and locomotor activity

To examine if the loss of function of the Magel2 gene in the ARCPOMC→MeA circuit regulates energy expenditure and locomotor activity, we performed indirect calorimetry on mice fed with HFD for 10 wk. Mice were individually housed in the calorimeter cages and acclimated to the respiratory chambers for at least 2 d before gas exchange measurements. Indirect calorimetry was performed for 5 d at the end of 10 wk using an open-circuit calorimetry system. O2 consumption and CO2 production were measured for each mouse at 9-min intervals over a 24-h period. The RER was calculated as the ratio of CO2 production over O2 consumption. Locomotor activity in X-Y and Z planes was measured by infrared beam breaks in the calorimetry cages. All data were analyzed with a Web-based Analysis Tool for Indirect Calorimetry Experiments CalR (Mina et al, 2018) (version 1.3, https://calrapp.org/). An ANCOVA analysis was performed to determine if there was a significant difference in energy expenditure between the groups.

Quantitative real-time PCR analysis

We collected ARC tissues from 9 AM to 10 AM without fasting. For qPCR analysis of the Magel2 gene, total RNAs were isolated using the RNeasy mini kit (74104; QIAGEN) from ARC tissues, and then first-strand cDNAs were synthesized using the SuperScript III First-Strand synthesis kit (18080-051; Thermo Fisher Scientific). Real-time qPCR was performed in sealed 96-well plates with SYBR Green I master mix (A25742; Applied Biosystems) using a Quant Studio 3 (Applied Biosystems). qPCR reactions were prepared in a final volume of 20 μl containing 2 μl cDNAs and 10 μl of SYBR Green master mix in the presence of primers at 0.5 μM. β-actin was used as an internal control for quantification of each sample. Amplification

Figure 6. Central ER-α does not contribute to the regulation of locomotor activity in female mice without the Magel2 gene in ARCPOMC neurons innervating the MeA.

(A) Pooled data showing plasma estradiol levels between the groups. Estradiol levels were higher in female mice without the Magel2 gene in ARCPOMC neurons innervating the MeA than in controls (POMCCre, n = 6 mice; POMCCre:Cas9-GFP, n = 7 mice). Two-tailed test, *P < 0.05. (B, C) Summary plots showing VO2 and respiratory exchange ratio between the experimental groups with ICI 182,780 infusion. At 10 wk of viral injection, the ER-α antagonist ICI 182,780 (40 ng/day) was infused into the LV via osmotic pumps at a rate of 0.25 μl/hour. (D, E) Pooled data showing total and ambulatory activity between the experimental groups with ICI 182,780 infusion. An increase in locomotor activity was still observed in mice without the Magel2 gene in ARCPOMC neurons innervating the MeA after treatment with ICI 182,780 (two-tailed t test, night, *P < 0.05; 24 h, *P < 0.05). (f, G, H) Summary plot showing TEE between the experimental groups. Regression plot showing TEE versus body weight (H).
was performed under the following conditions: denaturation at 95°C for 30 s, followed by 40 cycles of denaturation at 95°C for 30 s, and annealing/extension at 60°C for 1 min. The primers used for qPCR were the following: Magel2 forward, 5’-CAGCTCTCGGAGATGGTAAATG-3’ and reverse, 5’-AAAGGTGCACTCCAGCTTAG-3’; and β-actin forward, 5’-CCTCTATGCCAACACAGTGC-3’ and reverse, 5’-GCTAGGAGCCAGAGCAGTAA-3’. The relative expression levels were determined using the comparative threshold cycle (CT), which was normalized against the CT of β-actin using the ΔΔCt method.

**Immunofluorescence staining**

Mice were anesthetized with isoflurane (3%) and transectally perfused with pre-perfusion solution (9 g NaCl, 5 g sodium nitrate, Figure 7. Central GPER is critical in regulating locomotor activity in female mice without the Magel2 gene in ARCPOMC neurons innervating the MeA. (A) Images of confocal fluorescence microscopy showing expression of GPER in the VMH (middle) and MeA (right) in female mice. Scale bar: 100 μm. (B, C) Summary plots showing VO2 and respiratory exchange ratio between the experimental groups with G-15 infusion. At 10 wk of viral injection, the GPER antagonist G15 (5 μg/day [Mallet et al, 2021]) was infused into the LV via osmotic pumps at a rate of 0.25 μl/hour. There were no significant differences in VO2 and respiratory exchange ratio between the experimental groups. (D, E) Pooled data showing total and ambulatory activity between the experimental groups with G-15 infusion. An increase in locomotor activity was not observed in mice without the Magel2 gene in ARCPOMC neurons innervating the MeA following treatment with G-15 infusion. (F, G, H) Summary plot showing TEE between the experimental groups. Regression plot showing TEE versus body weight (H).
Measurement of plasma estradiol, testosterone, leptin, and insulin

Blood samples were collected from the retro-orbital plexus with heparinized capillary tubes (VWR International, LLC) and then centrifuged at 15,600g for 10 min at 4°C to collect plasma. Plasma estradiol, testosterone, leptin, and insulin levels were quantified using the ELISA kits (Cayman Chemical, 501890 for estradiol; Cayman Chemical, 582701 for testosterone; Thermo Fisher Technologies), Alexa 568 anti-rabbit IgG (1500, Cat. no. A10042; Life Technologies), Alexa 488 anti-mouse IgG (1:500, Cat. no. A21202; Life Technologies), and 5% bovine serum albumin for 2 h at room temperature and then incubated with Alexa 488 anti-mouse IgG (1:500, Cat. no. A21202; Life Technologies) for 2 h at room temperature. Tissues were washed, dried, and mounted with VECTASHIELD media containing DAPI. Images were acquired using a Leica SP8 confocal microscope. Cell counting was carried out with ImageJ software (version FIJI) as described in our previous work (Jeong et al, 2015, 2018; Kwon et al, 2020; Kwon & Jo, 2020). In brief, we used the Cell Counter plugin developed by Dr. Kurt De Vos (University of Sheffield). After initialization of the images, we manually counted MAGEL2+, GFP+, and MAGEL2/GFP+ cells in the ARC (250 × 250 μm).

Statistics

All statistical results are presented as mean ± SEM. Statistical analyses were performed using GraphPad Prism 9.0. Two-tailed t tests were used to calculate P-values of pair-wise comparisons. Time course comparisons between groups were analyzed using a two-way repeated-measures (RM) ANOVA with Sidak post hoc analysis. Differences were considered significantly different when the probability value was less than 0.05.

Supplementary Information

Supplementary Information is available at https://doi.org/10.26508/lsa.202201502.

Acknowledgements

We thank Drs. Patrick Potts and Klementina Fon Tacer for providing us with the MAGEL2 antibody and Drs. Gary Schwartz and Streamon Chua Jr. for their valuable feedback and comments on this study. We also thank Dr. Shun-Mei Liu and Licheng Wu for their technical assistance. This work was supported by the NIH (R01 DK092246, R01 AT011653, R03 TR003313, and P30 DK020541) and Foundation for Prader–Willi Research to Y-H Jo.

Author Contributions

Y Choi: data curation, software, formal analysis, investigation, and methodology.

Y-H Min: data curation, investigation, and methodology.

J Hwang: data curation, formal analysis, investigation, and methodology.

Y-H Jo: conceptualization, data curation, formal analysis, supervision, funding acquisition, validation, investigation, methodology, project administration, and writing—original draft, review, and editing.

Conflict of Interest Statement

The authors declare that they have no conflict of interest.

References

Alfaro DL, Lemoine P, Ehlinger V, Molinas C, Diene G, Valette M, Pinto G, Coupaye M, Poitou-Bernert C, Thuilleaux D, et al (2019) Causes of death in prader-willi syndrome: Lessons from 11 years' experience of a national reference center. Orphanet J Rare Dis 14: 238. doi:10.1186/s13023-019-1214-2

Baltašar N, Dalgaard LT, Lee CE, Yu J, Funahashi H, Williams T, Ferreira M, Tang V, McGovern RA, Kenny CD, et al (2005) Divergence of melanocortin pathways in the control of food intake and energy expenditure. Cell 123: 493–505. doi:10.1016/j.cell.2005.08.035

Biglari N, Gaziano I, Schumacher J, Radermacher J, Paeger L, Klemm P, Chen W, Corneliussen S, Wunderlich CM, Sue M, et al (2021) Functionally distinct pomic-expressing neuron subpopulations in hypothalamus revealed by intersectional targeting. Nat Neurosci 24: 913–929. doi:10.1038/s41593-021-00854-0

Bischof JM, Stewart CL, Wevrick R (2007) Inactivation of the mouse magel2 gene results in growth abnormalities similar to prader-willi syndrome. Hum Mol Genet 16: 2713–2719. doi:10.1093/hmg/ddm225

Boccaccio I, Glatt-Deeley H, Watrin F, Roeckel N, Lalande M, Muscatelli F (1999) The human magel2 gene and its mouse homologue are paternally expressed and mapped to the prader-willi region. Hum Mol Genet 8: 2407–2505. doi:10.1093/hmg/8.13.2497

Bumaschery VF, Yamashita M, Casas-Cordero R, Otero-Corcho V, de Souza FS, Rubinstein M, Low MJ (2012) Obesity-programmed mice are rescued by early genetic intervention. J Clin Invest 122: 4203–4212. doi:10.1172/jci62543

Burnett LC, LeDuc CA, Sulsouza CR, Paull D, Rausch R, Eddiry S, Carli JFM, Morabito MV, Skowronski AA, Hubner G, et al (2017) Deficiency in prohormone convertase pc1 impairs prohormone processing in prader-willi syndrome. J Clin Invest 127: 293–305. doi:10.1172/jci88648

Campbell JN, Macosko EZ, Fenselau H, Pers TH, Luybetskaya A, Tenen D, Goldman M, Verstegen AMJ, Resch JM, McCarroll SA, et al (2017) A molecular census of arcuate hypothalamus and median eminence cell types. Nat Neurosci 20: 484–496. doi:10.1038/nn.4495

Cassidy SB, Schwartz S, Miller JL, Driscoll DJ (2012) Prader-willi syndrome. Genet Med 14: 10–26. doi:10.1038/gim.0b013e318222bead0

Campbell JN, Macosko EZ, Fenselau H, Pers TH, Luybetskaya A, Tenen D, Goldman M, Verstegen AMJ, Resch JM, McCarroll SA, et al (2017) A molecular census of arcuate hypothalamus and median eminence cell types. Nat Neurosci 20: 484–496. doi:10.1038/nn.4495

Cassidy SB, Schwartz S, Miller JL, Driscoll DJ (2012) Prader-willi syndrome. Genet Med 14: 10–26. doi:10.1038/gim.0b013e318222bead0
Magel2 knockdown in hypothalamic POMC neurons
Choi et al.

Meyer MR, Barettella O, Prossnitz ER, Barton M (2010) Dilation of epicardial coronary arteries by the g-protein-coupled estrogen receptor agonists g-1 and icl 182, 780. Pharmacology 86: 58–64. doi:10.1159/00035497

Mina Al, LeClair RA, LeClair KB, Cohen DE, Lantier L, Banks AS (2018) Calr: A web-based analysis tool for indirect calorimetry experiments. Cell Metab 28: 656–666.e1. doi:10.1016/j.cmet.2018.06.019

Muscatelli F, Abrous DN, Massacrier A, Boccaccio I, Le Moal M, Cau P, Cremer H (2000) Disruption of the mouse necdn gene results in hypothalamic and behavioral alterations reminiscent of the human prader-willi syndrome. Hum Mol Genet 9: 3101–3110. doi:10.1093/hmg/9.20.3101

Nathan MR, Schmid P (2017) A review of fulvestrant in breast cancer. Oncol Ther 5: 17–29. doi:10.1016/s40487-017-0046-2

Qi Y, Purtell L, Fu M, Lee NJ, Aepler J, Zhang L, Loh K, Enriquez RF, Baldock PA, Resnick JL, Nicholls RD, Wevrick R (2013) Prader-Willi syndrome animal models working GRecommendations for the investigation of animal models of prader-willi syndrome. J Clin Invest 119: 2291–2303. doi:10.1172/jci37209

Shah BP, Vong L, Olson DP, Koda S, Krashes MJ, Ye C, Zhang JE, Lin S, Bao J, Wu P, Luo M, Zhan C (2015) Whole-brain mapping of the direct inputs and axonal projections of pomc and agrp neurons. Front Neuroanat 9: 40. doi:10.3389/fnana.2015.00040

Wei Q, Krolowski DM, Moore S, Kumar V, Li F, Martin B, Tomer R, Murphy GG, Deisseroth K, Watson SJ, et al (2018) Uneven balance of power between hypothalamic peptidegenic neurons in the control of feeding. Proc Natl Acad Sci U S A 115: E9489–E9498. doi:10.1073/pnas.1802237115

Wu MV, Manoli DS, Fraser EL, Coats JK, Tollkuhn J, Honda SI, Harada N, Shah NM (2009) Estrogen masculinizes neural pathways and sex-specific behaviors. Cell 139: 61–72. doi:10.1016/j.cell.2009.07.036

Xu P, Cao X, He Y, Zhu L, Yang Y, Saito K, Wang C, Yan X, Hinton AO, Jr, Zou F, et al (2015) Estrogen receptor-alpha in medial amygdala neurons regulates body weight. J Clin Invest 125: 2861–2876. doi:10.1172/jci80941

Xu Y, Nedungadi TP, Zhu L, Sobhani N, Irani BG, Davis KE, Zhang X, Zou F, Gent LM, Hahner LD, et al (2011) Distinct hypothalamic neurons mediate estrogenic effects on energy homeostasis and reproduction. Cell Metab 14: 453–465. doi:10.1016/j.cmet.2011.08.009

Xue B, Parnidimuakkala J, Lubahn DB, Hay M (2007) Estrogen receptor-alpha mediates estrogen protection from angiotensin ii-induced hypertension in conscious female mice. Am J Physiol Heart Circ Physiol 292: H1770–H1776. doi:10.1152/ajpheart.01011.2005

Yeoh GSH, Chao DHM, Siegert AM, Koerperich ZM, Ericson MD, Simonds SE, Larson CM, Luquet S, Clarke I, Sharma S, et al (2011) The melanocortin pathway and energy homeostasis: From discovery to obesity therapy. Mol Metab 48: 101206. doi:10.1016/j.molmet.2021.10.016

License: This article is available under a Creative Commons License (Attribution 4.0 International, as described at https://creativecommons.org/licenses/by/4.0/).