Neuroendocrinology Letters Volume 40 No. 5 2019
ISSN: 0172-780X; ISSN-L: 0172-780X; Electronic/Online ISSN: 2354-4716
Web of Knowledge / Web of Science: Neuroendocrinol Lett
Pub Med / Medline: Neuro Endocrinol Lett

REVIEW ARTICLE

Genes regulating the hypothalamic-pituitary--gonadal axis and its impact on pubertal onset in mammals

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Submitted: 2019-04-29 Accepted: 2019-07-12 Published online: 2019-12-12

Key words: **HPG axis; clinical genetics; puberty; kisspeptin; neurokinin**

Neuroendocrinol Lett 2019; **40**(5): 201–206 **PMID:** 32112543 NEL400519R01 • **www.nel.edu**

Abstract

Puberty in mammals is defined as the development of fertility, which involves the maturation of secondary sex characteristics and reproductive organs. This phenomenon is controlled by the Hypothalamic-Pituitary-Gonadal (HPG) axis. However, the timing of puberty differs greatly among individuals, and it is thought that a combination of genetic and environmental factors governs its onset. Advances in genetic analysis has allowed the identification of many more gene loci involved in regulating puberty. Understanding the genetics regulating the mammalian reproductive cycle can open novel therapeutic avenues for the treatment of human infertility. This review aims to explore the genes currently thought to regulate pubertal onset, in particular the KISS-1, and TAC-3 genes. Moreover, this review aims to provide insight into the emerging roles of the genes which usually regulate nutritional status including the FGF21 gene and how they may impact pubertal onset.

INTRODUCTION

Puberty in mammals is defined as the development of fertility, which involves the maturation of secondary sex characteristics and reproductive organs (Strauss & Barbieri 2009). The human reproductive system is controlled by the Hypothalamic-Pituitary-Gonadal (HPG) axis (Figure 1). Evidence suggests that pubertal onset is governed by a combination of genetic, nutritional and environmental factors via the HPG axis (Gajdos *et al.* 2008). Many genes have been identified as potential regulators of puberty. Ob (Ahima *et al.* 1997), *TAC3*, *TAC3R* (Topaloglu *et al.* 2008), KISS1 and KISS1R (Topaloglu *et al.* 2008) genes have been considered to influence pubertal onset, and recently studies have shown MKRN3 (Abreu *et al.* 2015) and FGF21 (Owen *et al.* 2013) genes are also involved in initiating puberty. Understanding the genetic mechanism behind the mammalian reproductive cycle could provide novel targets for the treatment of human infertility

Studies in mice (Wray *et al.* 1989a; Wray *et al.* 1989b) and rats (Yoshida *et al.* 1995) have shown that GnRH neurones migrate from the olfactory placode into the hypothalamus along the axons of the olfactory nervous system (Figure 2). The HPG-axis is active during foetal development, but around 6 months of gestation, GnRH secretion ceases and enters a period of relative quiescence until pubertal maturation (Terasawa & Fernandez 2000). This fluctuating pattern of GnRH activity suggests that pubertal onset may be controlled by inhibitory and stimulatory effects.

Since GnRH is involved in the maturation of gonads, genetic mechanisms which disrupt its

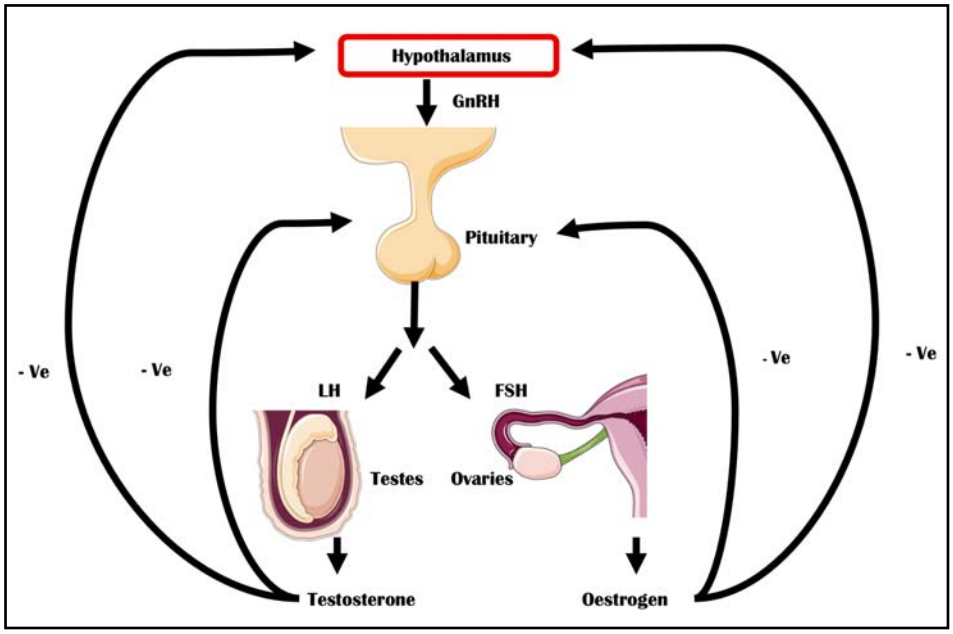

Fig. 1. HPG axis in males and females

Gonadotrophin-releasing hormone (GnRH) is released in a pulsatile manner from the hypothalamus into the hypophyseal portal circulation. GnRH binds to GnRH receptors on the anterior pituitary thereby initiating the secretion of luteinizing hormone (LH) and follicle stimulating hormone (FSH). LH and FSH result in secretion of testosterone from the testes in males and oestrogen from the ovaries in females. This axis is regulated by negative feedback.

migration pathway may alter GnRH activity and subsequently impact the pubertal process. The interference of this pathway is thought to play a role in the development of Idiopathic Hypogonadotropic Hypogandism (IHH). IHH is defined as either absent or delayed puberty, characterized by under developed gonads and low levels of serum sex steroids (Mitchell *et al.* 2011). Around 50% of IHH patients have anosmia (Quinton *et al.* 2001) due to absence of olfactory bulbs and tracts. The association between loss of olfaction and IHH is termed Kallmann's syndrome. Franco *et al.* (1991) showed that patients with Kallmann's syndrome have either partial or complete deletion of KALIG-1, a gene in the Xp22.3 region thought to be involved in neuronal migration. The finding from this investigation adds further evidence that genetic mutations interfering with the GnRH migration pathway result in the failure of pubertal onset.

REGULATING GNRH SECRETION: KISS1, KISS1R/GPR54R, AND MKRN3

The discovery of Kisspeptin and its receptor (KISSR/GPR54R) have played a pivotal role in shaping our understanding of pubertal regulation. Originally identified in 1996 as a metastasis-suppressor gene (15 the same as 14) (Franco *et al.* 1991), KISS1 encodes kisspeptin, which is a family of neuropeptide that binds to the KISS1 receptor. Studies in rodents have revealed that KISS1 mRNA is expressed in certain areas of the hypothalamus, including anteroventral periventricular nucleus (AVPV), the periventricular nucleus, and the arcuate nucleus(ARC) (Gottsch *et al.* 2004).

Le Roux *et al.* (Roux *et al.* 2003) isolated DNA from five IHH affected siblings of a large consanguineous family. Genomewide analysis revealed that these children had a defective GPR54 gene which impaired their pubertal development. The study concluded that the mutated GPR54 gene causes IHH by possibly disrupting the HPG axis. This finding is also supported by Seminara *et al.* (2003), who showed that GPR54-knockout mice had smaller reproductive tracts compared to wild type mice. The group also found that GnRH concentration in the hypothalamus did not differ significantly between the wild type and knockout mice. This finding suggests that the GPR54 does not alter the developmental biology of GnRH neurones, but rather kisspeptin may be involved in GnRH secretion. To investigate the influence of the GPR54 gene on the HPG axis, the group also administered intravenous GnRH to GPR54-deficient men, who initially had low concentration of LH and testosterone. Administration of GnRH led to an increase in LH levels, showing that GnRH acts downstream of GPR54.

Further research into the action of KISS-1 gene has suggested that kisspeptin may be responsible for the stimulation of GnRH secretion before the onset of puberty. Han and colleagues (Han *et al.* 2005) measured the amount of LH secreted by juvenile and adult mice in response to injections of kisspeptin into the lateral cerebral ventricle. The results showed that kisspeptin activated GnRH secretion more potently in adult mice. The group also conducted quantitative analysis

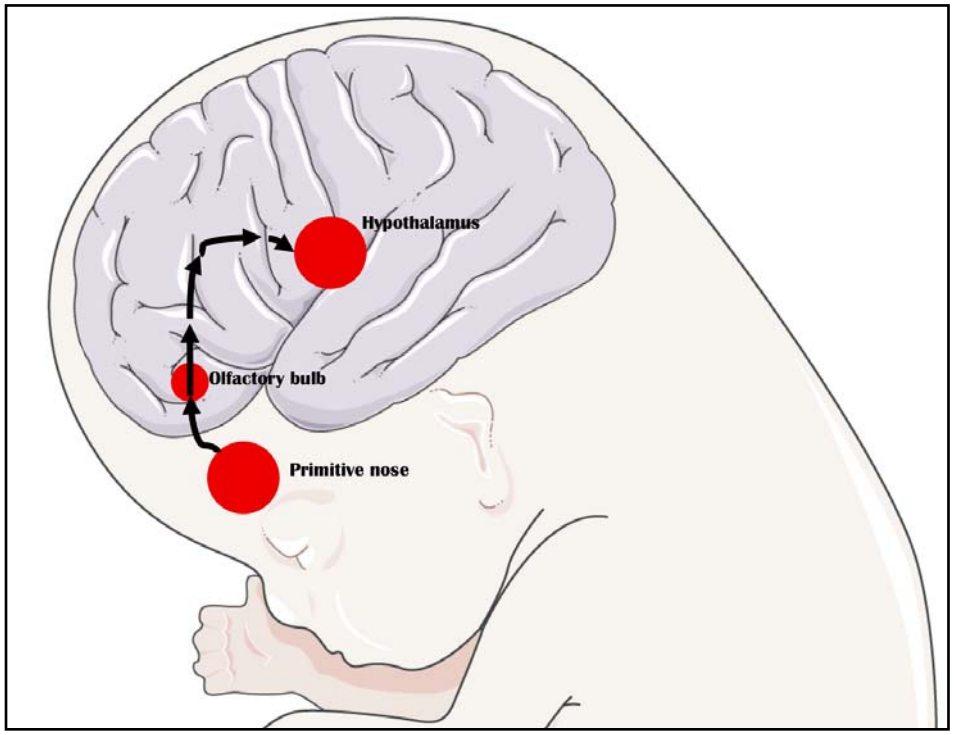

Fig. 2. Schematic diagram representing migration of GnRH neurones from olfactory placode into the forebrain

of kisspeptin mRNA expression in the AVPV and ARC nuclei, which revealed that both cell number (p <0.0001) and cell content (p <0.05) of kisspeptin increased from juvenile to adult mice in the AVPV. From this investigation, the group suggested that the sensitivity GnRH neurones to kisspeptin and kisspeptin input from AVPV increases over postnatal development.

Recent reports (Abreu *et al.* 2015: 2013) are highlighting the role of Marokin Ring Finger Protein 3 (MKRN3) in inhibiting GnRH secretion and influencing the onset of puberty. This peptide is encoded by the MKRN3 gene, which is in the same region on chromosome 15 as the mutated genes in Prader-Willi syndrome (PWS), a genetic condition characterized by abnormal growth development (Jong *et al.* 1999). Abreu *et al.* (2013) conducted real time polymerase-chain-reaction assays on male and female mice to evaluate the change in level of hypothalamic MKRN3 mRNA. The study revealed that levels of MKRN3 were high in the ARC nucleus of pre-pubertal mice, but rapidly decreased before the onset of puberty and remained low afterwards. The findings led the group to suggest that MKRN3 may have an inhibitory effect on GnRH secretion. To analyse the effects of circulating serum levels of MKRN3 in human puberty, Hagen and colleagues (Hagen *et al.* 2015) conducted a population-based longitudinal study of healthy Danish girls (n=38) and a prospective clinical study of early maturing girls (n=13). The study followed the healthy girls over 6 years and took blood samples every 6 months, measuring their serum MKRN3 concentration. Measurements of serum MKRN3 were also taken from early maturing girls. The results showed MKRN3 concentration declined before puberty began, and the girls who underwent early maturation had low levels of MKRN3, thus supporting the idea that the MKRN3 gene is an important regulator of GnRH secretion before puberty. A follow up longitudinal study (Busch *et al.* 2016) in healthy Danish boys has also shown the same trend. However, both of these studies had a small sample size, which reduces their statistical power. In addition, since the participants were all based in Denmark, further testing in different groups of people is required before reaching a conclusive answer.

Taken altogether, the findings suggest that KISS1 and MKRN3 genes are both important factors in the reactivation of GnRH before puberty. Further studies could investigate the mechanism by which MKRN3 inhibits GnRH secretion, and also explore whether there is an intermediate component which links the rise in kisspeptin levels and fall in MKRN3 before pubertal onset.

FURTHER GENETIC CONTROL OF GNRH RELEASE BY TAC3, TAC3R GENES

Another peptide thought to be implicated in GnRH secretion is Neurokinin (NKB). NKB and its receptor (NK3R) are encoded by the genes TAC3 and TACR3 respectively. NKB is co-expressed with kisspeptin neurones in the ARC nucleus (Goodman *et al.* 2007). Topaloglu (2008) *et al.* conducted a genomewide study on nine consanguineous Turkish families with IHH. They identified a mutation in NKB and NK3R in four of

those families thus proposing that NKB and its receptor are necessary for pubertal initiation. Since only Turkish families were recruited, the results could not be extrapolated to other groups of patients. However, in 2010, a much larger study was published by Gianetti *et al.* (2010), which included 18 families and 292 controls from different countries. The findings showed that the NKB pathway is involved in the development of hypogonadism, thus suggesting alteration in TAC3 and TACR3 genes could lead to a failure in regulating puberty.

The exact mechanism by which NKB influences the HPG axis has been greatly debated. Study on rats by Krajewski *et al.* (2005) suggested that NKB neurones stimulates GnRH secretion by interacting with NK3R in the median eminence. This conclusion is reinforced by Todman *et al.* (2005), who developed a profile of neurotransmitter receptors present on the GnRH neurones of mice, showing the presence TACR3 genes on the GnRH neruones. Oligonucleotide microarrays were used in this experiment to analyse the gene sequences present on the GnRH neurones, thus increasing the specificity of the procedure. However, recently Navarro *et al.* (2011) have contradicted these findings by reporting the absence of TACR3 mRNA on GnRH neurones. Since all of these investigations were conducted in rats and mice, there is no guarantee of any study producing the same results in humans.

GENES MONITORING NUTRITIONAL STATUS CAN ALSO INFLUENCE ONSET OF PUBERTY

Leptin, a product of the Ob gene is a peptide hormone synthesized and secreted by adipocytes (Hameed *et al.* 2010). The Ob gene is critical in energy homeostasis (Zhang *et al.* 1994) and neuroendocrine functions. Smith *et al.* (2006) demonstrated the co-expression of the leptin receptor (Ob-R) mRNA with KISS-1 mRNA in the ARC nucleus, suggesting leptin directly targets kisspeptin neurones. Further investigations on mice (Ingalls *et al.* 1950) and humans (Clément *et al.* 1998) have shown that the lack of Ob or Ob-R genes causes sterility and hypotrophic hypogonadism. Ahima and colleagues (Ahima *et al.* 1997) hypothesized leptin may be involved in determining pubertal timing. The investigation compared two groups of female mice by injecting one group with exogenous leptin and the other with saline. They monitored vaginal opening as a marker for pubertal onset. The results supported their hypothesis because vaginal opening occurred earlier in leptin-treated mice (29.2 +/- 0.3 days) compared with saline-treated controls (30.3 +/- 0.4 days). In this experiment, initiation of puberty was determined by vaginal opening, however in humans the onset of puberty is a much more complex process involving behavioral, physical and hormonal changes. In addition, the group administered recombinant leptin to mice, therefore it cannot be assumed that endogenous leptin will have the same physiological effect in humans.

To investigate the effects of leptin in controlling human puberty, Mantzoros *et al.* (1997) conducted a longitudinal assessment on eight pre-pubertal boys. The team hypothesized that leptin is an important peptide responsible for triggering the onset of puberty. The boys were either in Tanner genital stage 1 or early stage 2 at the start of the trial, and were evaluated over 2.1-5.1 years depending on when Tanner genital stage 5 was reached. Every four months, their serum leptin, testosterone and dehydroepiandrosterone sulfate (DHEAS) were measured. The study found that leptin levels rose

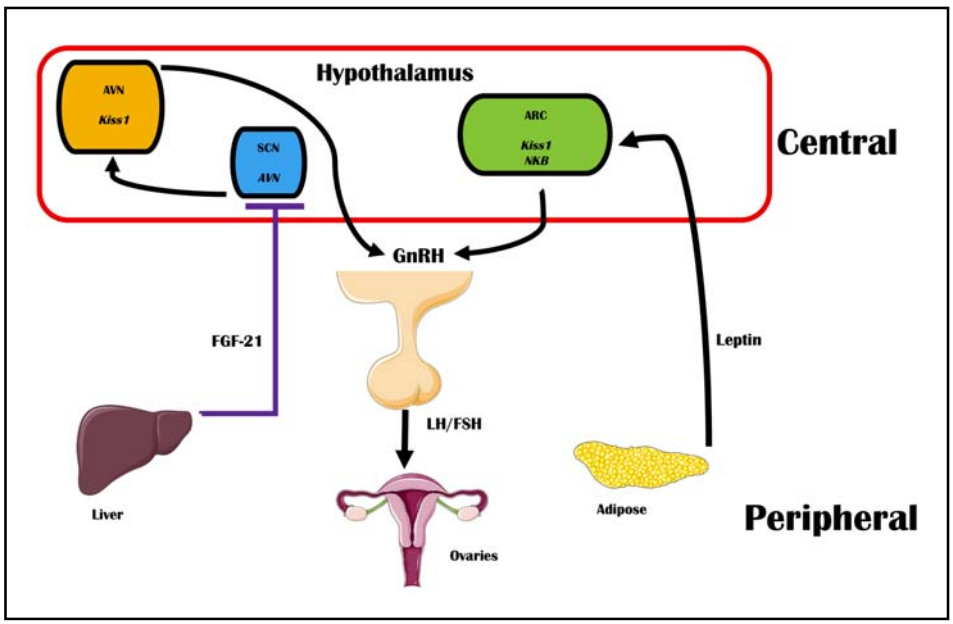

Fig. 3. Schematic diagram representing central and peripheral mechanisms of GnRH secretion.
Note: Negative feedback is not shown here.

by 50% compared to the baseline perpubertal levels just before the onset of puberty (P< 0.01). However, there was no periodic measurement of other variables which could influence leptin concentration such as Insulin, which is considered to have a positive effect on leptin levels (Saladin *et al.* 1995) or adrogenic agonists, which are thought to act negatively (Mantzoros *et al.* 1996). Therefore, despite the positive trend identified in this study between puberty initiation and leptin levels, the results are not completely conclusive.

Another advancement, which has offered insights into the mechanisms underlying the onset of puberty is the action of Fibroblast growth factor 21 (FGF21) on the hypothalamus. FGF21 is a peptide encoded by the FGF21 gene, which is activated in the liver to regulate carbohydrate and fatty acid metabolism during fasting (Potthoff *et al.* 2009). It has been shown that FGF21 requires its co-receptor β-klotho to be fully functional. (Ding *et al.* 2012)

In a recent study, Owen *et al.* (2003) observed that transgenic female mice overexpressing FGF21, tg(FGF21,) were infertile compared to control wild type mice and took much longer to start puberty. Cytology of these transgenic mice revealed they lacked corpa leutea. The tg(FGF21) mice also had significantly lower levels of LH compared to the wild types and normal FSH profile suggesting these mice had hypogonadotrophic hypogonadism. Further stimulation and hormonal analysis of the HPG axis revelaed that the tg(FGF21) mice had a problem at the hypothalamic level.

Study conducted in mice by Vida *et al.* (2010) demonstrate that KISS1 neurones in the AVPV nuclei receive signal from the suprachiasmatic nucleus (SCN) via vasopressinergic neruones. Based on these findings, Owen and colleagues (Owen *et al.* 2013) evaluated the action of FGF21 on AVPV and SCN by measuring gene expression of KISS1 in AVPV and vasopressin (AVP) in the SCN. Analysis showed that gene expression of KISS1 and AVP was significantly reduced in the tg(FGF21) mice compared to the wild type. Further investigations comparing tg(FGF21) mice with β-klotho deficient tg(FGF21) mice, revealed higher gene expression of KISS1 in the AVPV and AVP in the SCN by the latter group. Together, these series of investigations show that FGF21 acts on the SCN via β-klotho FGF co-receptors to inhibit kisspeptin neurons in the AVPV through the vasopressenergic pathway. Whilst this study was very systematic in its approach, the same methods cannot be applied in humans. In particular, the group reported that the location of AVPV nuclei and SCN were determined in relation to white matter landmarks and the third ventricle. However, in humans there is no structural presence of the AVPV, hence these methodologies are restricted to rodent models.

PERSPECTIVE AND CONCLUDING REMARKS

The onset of puberty is governed by many genes, which encode different peptides that are located both centrally and peripherally (Figure 3). These peptides influence puberty by acting at various locations on the HPG axis. Knowledge of the mechanisms by which these peptides interact may create new targets for the treatment of fertility. Further investigations are needed to explore how the function of these genes is associated with environmental factors to govern pubertal onset.

REFERENCES

1 Abreu A, Dauber A, Macedo D, Noel S, Brito V, Gill J, Cukier P, Thompson I et al. (2013). Central precocious puberty caused by mutations in the imprinted gene MKRN3. *The New England journal of medicine.* **368**(26): 2467–2475.
2 Abreu A, Macedo D, Brito V, Kaiser U, Latronico A (2015). A new pathway in the control of the initiation of puberty: The MKRN3 gene. *Journal of molecular endocrinology.* **54**(3).
3 Ahima R, Dushay J, Flier S, Prabakaran D, Flier J (1997). Leptin accelerates the onset of puberty in normal female mice. *The Journal of clinical investigation.* **99**(3): 391–395.
4 Busch A, Hagen C, Almstrup K, Juul A (2016). Circulating MKRN3 levels decline during puberty in healthy boys. *The Journal of clinical endocrinology and metabolism.* **101**(6): 2588–2593.
5 Clément K, Vaisse C, Lahlou N, Cabrol S, Pelloux V, Cassuto D, Gourmelen M, Dina C et al. (1998). A mutation in the human leptin receptor gene causes obesity and pituitary dysfunction. *Nature.* **392**(6674): 398–401.
6 Ding X, Boney-Montoya J, Owen BM, et al. (2012). βKlotho Is Required for Fibroblast Growth Factor 21 Effects On Growth and Metabolism. *Cell metabolism.* **16**(3): 387–393.
7 Franco B, Guioli S, Pragliola A, Incerti B, Bardoni B, Tonlorenzi R, Carrozzo R, Maestrini E et al. (1991). A gene deleted in Kallmann's syndrome shares homology with neural cell adhesion and axonal path-finding molecules. *Nature.* **353**(6344): 529–536.
8 Gajdos Z, Hirschhorn J, Palmert (2008). What controls the timing of puberty? An update on progress from genetic investigation. *Current opinion in endocrinology, diabetes, and obesity.***16**(1): 16–24.
9 Gianetti E, Tusset C, Noel S, Au M, Dwyer A, Hughes V, Abreu A, Carroll J et al. (2010). TAC3/TACR3 mutations reveal preferential activation of gonadotropin-releasing hormone release by neurokinin B in neonatal life followed by reversal in adulthood. *The Journal of clinical endocrinology and metabolism.* **95**(6): 2857–2867.
10 Goodman R, Lehman M, Smith J, Coolen L, Oliveira de, Jafarzadehshirazi, Pereira A, Iqbal J et al. (2007). Kisspeptin neurons in the arcuate nucleus of the ewe express both dynorphin A and neurokinin B. *Endocrinology.* **148**(12): 5752–5760.
11 Gottsch M, Cunningham M, Smith J, Popa S, Acohido B, Crowley W, Seminara S, Clifton et al. (2004). A role for kisspeptins in the regulation of gonadotropin secretion in the mouse. *Endocrinology.* **145**(9): 4073–4077.
12 Hagen C, Sørensen K, Mieritz M, Johannsen T, Almstrup K, Juul A (2015). Circulating MKRN3 levels decline prior to pubertal onset and through puberty: A longitudinal study of healthy girls. *The Journal of clinical endocrinology and metabolism.* **100**(5): 1920–1926.
13 Hameed S, Jayasena C, Dhillo W (2010). Kisspeptin and fertility. *The Journal of endocrinology.* **208**(2): 97–105.

14 Han S, Gottsch M, Lee K, Popa S, Smith J, Jakawich S, Clifton D, Steiner R et al. (2005). Activation of gonadotropin-releasing hormone neurons by kisspeptin as a neuroendocrine switch for the onset of puberty. *The Journal of neuroscience : the official journal of the Society for Neuroscience.* **25**(49): 11349–11356.
15 Ingalls A, Dickie M, Snell G (1950). Obese, a new mutation in the house mouse. *The Journal of heredity.* **41**(12): 317–318.
16 Jong M, Gray T, Ji Y, Glenn C, Saitoh S, Driscoll D, Nicholls R (1999). A novel imprinted gene, encoding a RING zinc-finger protein, and overlapping antisense transcript in the Prader-Willi syndrome critical region. *Human molecular genetics.* **8**(5): 783–793.
17 Krajewski S, Anderson M, Iles-Shih L, Chen K, Urbanski H, Rance N (2005). Morphologic evidence that neurokinin B modulates gonadotropin-releasing hormone secretion via neurokinin 3 receptors in the rat median eminence. *The Journal of comparative neurology.* **489**(3): 372–386
18 Mantzoros C, Flier J, Rogol A (1997). A longitudinal assessment of hormonal and physical alterations during normal puberty in boys. V. Rising leptin levels may signal the onset of puberty. *The Journal of clinical endocrinology and metabolism.* **82**(4): 1066–1070.
19 Mantzoros C, Qu D, Frederich R, Susulic V, Lowell B, Maratos-Flier E, Flier J (1996). Activation of beta(3) adrenergic receptors suppresses leptin expression and mediates a leptin-independent inhibition of food intake in mice. *Diabetes.* **45**(7): 909–914.
20 Mitchell A, Dwyer A, Pitteloud N, Quinton R (2011). Genetic basis and variable phenotypic expression of Kallmann syndrome: Towards a unifying theory. *Trends in endocrinology and metabolism: TEM.* **22**(7): 249–258.
21 Navarro V, Gottsch M, Wu M, García-Galiano D, Hobbs S, Bosch M, Pinilla L, Clifton D et al. (2011). Regulation of NKB pathways and their roles in the control of kiss1 neurons in the arcuate nucleus of the male mouse. *Endocrinology.* **152**(11): 4265–4275.
22 Owen B, Bookout A, Ding X, Lin V, Atkin S, Gautron L, Kliewer S, Mangelsdorf D (2013). FGF21 contributes to neuroendocrine control of female reproduction. *Nature medicine.* **19**(9): 1153–56.
23 Potthoff MJ, Inagaki T, Satapati S, et al. (2009). FGF21 induces PGC-1α and regulates carbohydrate and fatty acid metabolism during the adaptive starvation response. *Proceedings of the National Academy of Sciences of the United States of America.* **106**(26): 10853–10858.
24 Quinton R, Duke V, Robertson A, Kirk J, Matfin G, Zoysa de, Azcona C, MacColl G et al. (2001). Idiopathic gonadotrophin deficiency: Genetic questions addressed through phenotypic characterization. *Clinical endocrinology.* **55**(2): 163–174.
25 Roux de, Genin E, Carel J, Matsuda F, Chaussain J, Milgrom E (2003). Hypogonadotropic hypogonadism due to loss of function of the kiSS1-derived peptide receptor GPR54. *Proceedings of the National Academy of Sciences of the United States of America.* **100**(19): 10972–10976.
26 Saladin R, Vos D, Guerre-Millo M, Leturque A, Girard J, Staels B, Auwerx J (1995). Transient increase in obese gene expression after food intake or insulin administration. *Nature.* **377**(6549): 527–529.
27 Seminara S, Messager S, Chatzidaki E, Thresher R, Acierno J, Shagoury J, Bo-Abbas Y, Kuohung W et al. (2003). The GPR54 gene as a regulator of puberty. *The New England journal of medicine.* **349**(17): 1614–2167.
28 Smith J, Acohido B, Clifton D, Steiner R (2006). KiSS-1 neurones are direct targets for leptin in the ob/ob mouse. *Journal of neuroendocrinology.* **18**(4): 298–303.
29 Strauss J, Barbieri R (2009). *Yen and Jaffe's Reproductive Endocrinology.* Sixth Edition. Philadelphia; Saunders Elsevier.
30 Terasawa E, Fernandez D (2000). Neurobiological mechanisms of the onset of puberty in primates. *Endocrine reviews.* **22**(1):111–151.
31 Todman M, Han S, Herbison A (2005). Profiling neurotransmitter receptor expression in mouse gonadotropin-releasing hormone neurons using green fluorescent protein-promoter transgenics and microarrays. *Neuroscience.* **132**(3): 703–712.
32 Topaloglu A, Reimann F, Guclu M, Yalin A, Kotan L, Porter K, Serin A, Mungan N et al. (2008). TAC3 and TACR3 mutations in familial hypogonadotropic hypogonadism reveal a key role for Neurokinin B in the central control of reproduction. *Nature genetics.* **41**(3): 354–358.
33 Vida B, Deli L, Hrabovszky E, Kalamatianos T, Caraty A, Coen C, Liposits Z, Kalló I (2010). Evidence for suprachiasmatic vasopressin neurones innervating kisspeptin neurones in the rostral periventricular area of the mouse brain: Regulation by oestrogen. *J Neuroendocrinology.* **22**(9): 1032–1039.
34 Wray S, Grant P, Gainer H (1989a). Evidence that cells expressing luteinizing hormone-releasing hormone mRNA in the mouse are derived from progenitor cells in the olfactory placode. *Proceedings of the National Academy of Sciences of the United States of America.* **86**(20): 8132–8136.
35 Wray S, Nieburgs A, Elkabes S (1989b). Spatiotemporal cell expression of luteinizing hormone-releasing hormone in the prenatal mouse: Evidence for an embryonic origin in the olfactory placode. *Brain research. Developmental brain research.* **46**(2): 309–318.
36 Yoshida K, Tobet S, Crandall J, Jimenez T, Schwarting G (1995). The migration of luteinizing hormone-releasing hormone neurons in the developing rat is associated with a transient, caudal projection of the vomeronasal nerve. *The Journal of neuroscience: the official journal of the Society for Neuroscience.* **15**(12): 7769–7777.
37 Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman J (1994). Positional cloning of the mouse obese gene and its human homologue. *Nature.* **372**(6505): 425–432.