NIH Public Access
Author Manuscript
Adv Exp Med Biol. Author manuscript; available in PMC 2014 February 17.

Published in final edited form as:
Adv Exp Med Biol. 2013 ; 784: 187–199. doi:10.1007/978-1-4614-6199-9_9.

Kisspeptin and Clinical Disorders

Letícia G. Silveira[1], **Ana Claudia Latronico**[1], and **Stephanie B. Seminara**[2]

[1]Unidade de Endocrinologia do Desenvolvimento, Laboratório de Hormônios e Genética Molecular/LIM42, Hospital das Clinicas da Faculdade de Medicina da Universidade de São Paulo, São Paulo, Brasil

[2]Reproductive Endocrine Unit, Department of Medicine, Massachusetts General Hospital, 55 Fruit Street, Boston, MA 02114, U.S.A

Abstract

The hypothalamic hormone GnRH has traditionally been viewed as a central driver of the hypothalamic-pituitary-gonadal axis. Pulsatile GnRH release is required for pulsatile gonadotropin secretion, which then modulates gonadal steroid feedback, and brings about full fertility in the adult. Pathways governing GnRH ontogeny and physiology have been discovered by studying humans with disorders of GnRH secretion. In this chapter, the human genetics of the kisspeptin signaling pathway in patients with diverse reproductive phenotypes will be explored. The discovery of defects in the kisspeptin system in several reproductive disorders has shed light on the mechanisms involved in regulating GnRH secretion, revealing the critical role played by the kisspeptin signaling pathway in pubertal initiation and reproductive function.

GnRH Deficiency

GnRH deficiency is a condition characterized by abnormal pubertal development and low gonadotropins and sex steroids. Administration of exogenous pulsatile GnRH long-term can restore normal levels of gonadotropins and sex steroids in patients with this disorder, demonstrating the hypothalamic nature of the defect in the vast majority of patients [1,2]. GnRH deficiency is heterogenous in its clinical presentation, with variation in the presence or absence of olfactory defects (and other somatic anomalies), severity of the hypogonadism, and neuroendocrine patterns. Thus, patients with isolated GnRH deficiency represent a unique opportunity to identify genes that awaken the reproductive cascade at the time of sexual maturation and maintain normal reproductive function throughout life.

In 2003, homozygosity mapping and candidate gene analysis of two large consanguineous pedigrees with isolated GnRH deficiency led to the identification of loss of function mutations in a then little-known G protein-coupled receptor, *GPR54* (later to be renamed *KISS1R* = kisspeptin receptor), by two investigative groups [3,4]. The identification of mutations in multiple families, as well as unrelated probands, coupled with a parallel reproductive phenotype in a *Kiss1r* mutant mouse, catapulted kisspeptin into the spotlight as a key regulator of GnRH secretion.

Initial Reports

In searching for novel gene defects associated with isolated GnRH deficiency, one group employed homozygosity mapping of a large consanguineous family with 5 affected siblings.

Address all correspondence to: Stephanie Seminara MD Massachusetts General Hospital BHX 504 Fruit Street Boston MA 02114 sseminara@partners.org.

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Chromosome localization and candidate gene sequence analysis led to the identification of a homozygous deletion of 155 nucleotides in the *KISS1R* [3]. This deletion encompassed the splicing acceptor site of intron 4-exon 5 junction and part of exon 5. In the unlikely event that this abnormal transcript was translated, the deleted receptor would be truncated within the third intracellular loop, lacking transmembrane domains 6 and 7. The proband of the index family was a 20 year old male who presented with abnormal pubertal development, 4 ml testes and a normal sense of smell. While his affected brothers all had similar clinical features, his affected sister had partial breast development, and had experienced a single episode of uterine bleeding. His mother, a heterozygote carrier of the deletion, was noted to have experienced menarche at age 16.

Homozygosity mapping of a different consanguineous family with GnRH deficiency, this time from the Middle East, was the focus of a second investigative group [4]. Candidate gene sequencing of *KISS1R* led to the discovery of a homozygous missense mutation, p.L138S, within the second intracellular loop of the receptor. Transfection of COS-7 cells with a mutant construct representing L148S revealed significantly decreased accumulation of inositol phosphate *in vitro* compared to wild type [4]. Further sequencing of *KISS1R* led to the identification of compound heterozygote mutations, p.R331X and p.X399R, in an African American male [4]. As mRNAs with premature termination codons are known to be subject to nonsense-mediated decay [5], and mRNAs without an in-frame termination codon had recently been appreciated to be subject to nonstop decay [6,7], it was hypothesized that the combination of nonstop and nonsense mutations in a single individual would result in the absence of a functional receptor. Quantitative RT-PCR confirmed a significant reduction of *KISS1R* mRNA in immortalized white blood cells from the p.R331X/p.X399R proband. Should a protein have been produced by either the p.R331X or p.X399R transcripts, *in vitro* studies suggested that each of the mutant proteins would have functioned poorly.

Understanding the functional consequences of the p.R331X/p.X399R mutations was an important backdrop for the interpretation of the clinical presentation of the p.R331X/p.X399R proband [4]. On frequent blood sampling, this individual had low-amplitude pulses of luteinizing hormone (LH), suggesting present, but enfeebled, secretion of GnRH. He also had a left-shifted dose–response curve compared to other patients with GnRH deficiency undergoing the same therapy, suggesting that he may be more sensitive to exogenous GnRH. Thus, despite the clear importance of the kisspeptin pathway in modulating GnRH release, the clinical data from this patient suggested the possibility of residual GnRH activity, a foreshadowing of subsequent studies that were performed in the rodent demonstrating the existence of kisspeptin-independent GnRH secretion [8].

The clinical presentations of the p.L148S and p.R331X/p.X399R patients were also juxtaposed against the phenotype of *Kiss1r* deficient mice; the mutant mice were striking phenocopies of the GnRH deficient patients, including lack of sexual maturation associated with low levels of gonadotropins [4]. The strong parallels in presentation between the GnRH deficient patients and the mutant mice established a central role for *KISS1R/Kiss1r* across mammalian species. Moreover, the use of a mouse model allowed quantification of hypothalamic GnRH content, which was found to be normal in the *Kiss1r* deficient mice, suggesting that *KISS1R/Kiss1r* influences the timing of sexual maturation by affecting the processing or secretion of GnRH [4,9].

Thus, the identification of mutations in *KISS1R* by multiple groups thrust the kisspeptin pathway into the spotlight, and laboratories around the world began assembling expression, physiologic, transgenic, knock out/down, and electrophysiologic data to tell the biologic story of kisspeptin and its important role in regulating GnRH secretion. The hypothesis that kisspeptin does not affect GnRH neuronal migration, but rather GnRH biosynthesis and/or

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

release, was supported shortly thereafter by *in vivo* studies demonstrating that kisspeptin administration, either centrally or peripherally, triggers robust GnRH-induced LH and FSH secretion [10,11]. Kisspeptin expression in the hypothalamus of rodents and nonhuman primates was also found to increase at the time of sexual maturation [11,12], an important finding since kisspeptin is thought to mediate sex steroid feedback [12-17], estrous cycle regulation [17,18], seasonal breeding [19-25], and to convey information about the energy status of the organism [26,27]. Thus, relatively quickly, kisspeptin was found to be a key gatekeeper for the activation of the GnRH axis.

Central Themes for Patients Carrying Mutations in the Kisspeptin Signaling Pathway

Once *KISS1R* was added to the roster of genes for GnRH deficiency, several groups began to search for mutations in the kisspeptin signaling pathway in patients with hypogonadotropic hypogonadism. However, mutations in the coding sequence of *KISS1R* have proven to be relatively rare, particularly in comparison with many other genes for GnRH deficiency; for example, *KAL1* has been reported to harbor mutations in 5-14% of GnRH deficient patients [28-30], considerably higher than the 1% frequency of mutations in *KISS1R* [28]. It is unclear why the prevalence of mutations in the kisspeptin signaling pathway is relatively low, but it is possible that kisspeptin's role in trophoblast invasion [31,32] or metastasis suppression [33,34] creates negative selection pressure against the promulgation of mutations in families.

Reported biallelic mutations of *KISS1R* include a homozygous 155 base pair deletion [3], a homozygous frameshift (c.1001_1002insC) [35], a homozygous splice acceptor site mutation [36], homozygous p.L102P [37], p.L148S [4,38,39] and p.F272S missense mutations [40], and the compound heterozygous mutations p.R331X/p.X399R [4,39] and p.R297L/p.C223R [41]. Thus, mutations in this G protein-coupled receptor are variable in type (large deletion, frameshift, splice site, nonsense, nonstop, and missense) and occur throughout the receptor.

Typically, patients carrying biallelic complete loss-of-function mutations serve as the "phenotypic bookends" of the most extreme clinical presentation that can be associated with loss of a particular gene. In general, complete loss of kisspeptin signaling is associated with normosmic GnRH deficiency but, as noted earlier, the abnormalities in GnRH secretion may be partial. For example, an affected female carrying a homozygous 155 base pair deletion in *KISS1R* (that if translated, would lead to a truncated G protein-coupled receptor unable to stimulate the transduction pathway) presented with some partial breast development and one episode of uterine bleeding [3]. Although her sexual maturation was clearly abnormal, her breast development and uterine bleeding suggest her endogenous estradiol levels were above prepubertal values. Another female, this time carrying a homozygous L102P mutation, demonstrated a robust LH level in response to a 100μg GnRH stimulation test (peak approximately 32 IU/L) at initial evaluation [37]. During 6 hours of frequent blood sampling, she manifested low amplitude LH pulses occurring approximately once per hour. These clinical clues all suggest the presence of some degree of enfeebled endogenous GnRH secretion.

To date, patients with biallelic mutations in *KISS1R* lack the syndromic features that can be found in anosmic forms of this disorder (Kallmann syndrome), including cleft lip and palate, synkinesia, and renal agenesis. This is likely due to the fact that mutations in the kisspeptin signaling pathway do not affect olfactory bulb development and by extension, GnRH neuronal migration, a hypothesis supported by studies of *Kiss1r* $^{-/-}$ mice, which have normal GnRH hypothalamic content and preserved anatomy [4,55,56]. However, because

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

GnRH deficiency can be an oligogenic disease [28], it is possible that patients with Kallmann syndrome carry mutations in genes that affect not only GnRH neuronal migration but also kisspeptin signaling [42].

Discovery of Mutations in Closely Related Pathways

Kisspeptin is now appreciated to be co-expressed with other neuropeptides that are likely to work in a cooperative fashion to regulate the hypothalamic control of reproduction. Kisspeptin neurons in the ARC co-express the neuropeptides neurokinin B (NKB) and dynorphin, giving rise to the term KNDY neurons (Kisspeptin-Neurokinin B-Dynorphin); this co-localization has been observed in several mammalian species, including humans [43-45]. About five years after the discovery of mutations in the kisspeptin pathway, loss-of-function mutations in the genes encoding neurokinin B (*TAC3*) and its receptor (*TACR3*) in patients with normosmic isolated hypogonadotropic hypogonadism (IHH) and pubertal failure were discovered [46]. Patients with mutations in *KISS1R* have a relatively straightforward phenotype without syndromic features, although there can be evidence of residual GnRH activity [3,4,37]. Patients bearing mutations in the neurokinin B signaling pathway also lack anosmia, renal agenesis, and bony abnormalities, but their neuroendocrine phenotype is more complex. A large proportion of patients with mutations in either *TAC3* or *TACR3* have undergone reversal of their hypogonadotropism [47], a phenomenon in which patients undergo spontaneous recovery of their hypothalamic-pituitary-gonadal cascade. *Tacr3*$^{-/-}$ mice have numerous reproductive defects (including abnormal estrous cycles, reduced corpora lutea, decreased uterine weights) but mutant female mice are able to achieve fertility when mated, demonstrating that these mice are more similar to the patients with *TAC3/TACR3* mutations than previously appreciated [48]. While reversible GnRH deficiency is not exclusively associated with *TAC3/TACR3* [49-54], none of the patients bearing biallelic mutations in the kisspeptin signaling pathway have yet to be reported with this clinical sub-phenotype. While the triggers to reversal remain poorly understood, these clinical observations appear to be providing important clues as to the physiologic hierarchy and relative influence of neurokinin B and kisspeptin in modulating GnRH release. In fact, the possibility that the actions of neurokinin B are proximal to those of kisspeptin is supported by the observation of increased LH pulse frequency during kisspeptin infusion to patients with neurokinin B signaling deficiencies [55].

Fertility Phenotypes

Although fertility data is available on only a subset of patients, mutations in *KISS1R* do not appear to impact fertility potential. Despite bilateral cryptorchidism and mild hypospadius, a male patient carrying a homozygous *KISS1R* c.1001_1002insC mutation responded to exogenous pulsatile GnRH, normalizing testosterone levels and inducing spermatogenesis [35]. Because his semen analysis showed oligoasthenozoospermia, pregnancy, albeit achieved with assisted reproduction, was possible. The male proband harboring p.R331X/p.X399R also received pulsatile GnRH and experienced steady increases in testicular volume and the appearance of normal spermatogenesis, resulting in fertility [39]. A homozygous p.L148S female had 1) intact responses to exogenous GnRH and gonadotropins, 2) multiple conceptions using the aforementioned therapies as well as IVF, and 3) two uncomplicated pregnancies [39]. While details are not available regarding the quality of her follicular response, mutations in *KISS1R* do not appear to preclude steroidogenesis and gametogenesis.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

The Missing Link of KISS1

In GnRH deficiency, the discovery of loss-of-function mutations in genes encoding cell membrane-associated receptors has always preceded or accompanied the discovery of disabling mutations in the genes encoding their ligands. Initially, it appeared that *KISS1* was going to escape this genetic tradition, as no mutations in *KISS1* were reported for 8 years after the discovery of mutations in *KISS1R*. However, mutations in *KISS1* are now clearly associated with GnRH deficiency.

In 2011, 15 probands with GnRH deficiency were found to harbor 10 heterozygous rare sequence variants in *KISS1* [56]; *in silico*, *in vitro*, and *in vivo* studies were performed to explore the functional consequences of these variants. p.F117L was found to reduce inositol phosphate generation *in vitro*. p.G35S and p.C53R were predicted *in silico* to be deleterious. Lying outside the coding region, the variant g.1-3659C→T was found to impair transcription *in vitro* while another variant, c.1-7C→T, was noted to sit within the consensus Kozak sequence. Because these variants were monoallelic, and not biallelic, an examination of reproductive phenotypes in heterozygous and double-heterozygous *Kiss1* and *Kiss1r* mice was also performed. Heterozygous *Kiss1* mutations produced reproductive phenotypes in mutant mice and these phenotypes were further accentuated when accompanied by heterozygous mutations in *Kiss1r* [56].

As over 1000 probands were screened to identify these nucleotide changes, disabling genetic variation in *KISS1* is clearly as rare, if not more so, than that of *KISS1R*. However, a homozygous loss-of-function mutation in *KISS1* was eventually discovered in affected siblings from a consanguineous Kurdish pedigree with normosmic GnRH deficiency (c. 345C→G; p.N115K) [57]. The mutant kisspeptin was significantly less potent than wild type kisspeptin in activating GnRH neurons. Thus, both *KISS1R* and *KISS1* are clearly genetic determinants of the timing of sexual maturation in the human.

Insights Garnered from Kiss1$^{-/-}$ and Kiss1r$^{-/-}$ Mice

In general, *Kiss1*$^{-/-}$ and *Kiss1r*$^{-/-}$ mice are phenocopies of humans bearing *KISS1R* mutations. Both *Kiss1*$^{-/-}$ and *Kiss1r*$^{-/-}$ mice have small gonads, low gonadotropins, and abnormal gametogenesis, and infertility [4,9,58,59]. GnRH neuronal migration into the hypothalamus is normal in *Kiss1*$^{-/-}$ animals, along with appropriate axonal connections to the median eminence and total GnRH content [58].

Despite their infertility, *Kiss1*$^{-/-}$ female mice can develop follicles, up to the pre-ovulatory level, although no spontaneous ovulations are observed [8]. Both *Kiss1*$^{-/-}$ and *Kiss1r*$^{-/-}$ females alternate between periods of prolonged diestrus and prolonged estrus. These transitions increase in frequency with increasing age and are not associated with changes in hypothalamic *Gnrh1* mRNA expression. Administration of the competitive GnRH antagonist acyline disrupts the estrus exhibited by *Kiss1*$^{-/-}$ and *Kiss1r*$^{-/-}$ female mice, demonstrating that this estrus is due to GnRH activity. The low amplitude LH pulsations observed in multiple patients with *KISS1R* mutations appears to be echoed in the persistent GnRH activity documented in *Kiss1*$^{-/-}$ and *Kiss1r*$^{-/-}$ mice. Kisspeptin-independent GnRH activity, whether in mice or men, could be due to low level constitutive activity of GnRH neurons or could be induced by other neuroendocrine pathways that modulate GnRH neuronal secretion.

Surprisingly, mice with targeted ablation of kisspeptin or kisspeptin receptor expressing *cells* (as opposed to deletion of the *Kiss1* and *Kiss1r genes*) are almost entirely reproductively normal suggesting, at initial interpretation, that kisspeptin signaling is not required for puberty and fertility [60]. However, the residual kisspeptin and GnRH neurons

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

present in each of these cellular ablation mouse models, while quite small in number, may in fact be sufficient for sexual maturation and fertility, as initially suggested by preoptic area brain grafts in hypogonadal (*hpg*) mice [61,62] and a more recent mouse model (*Kiss* $^{Cre/Cre}$) with markedly reduced expression of *Kiss1* [63].

From Loss-of-Function to Gain-of-Function in the Kisspeptin Signaling Pathway

While GnRH deficiency presents with delayed pubertal development, central precocious puberty (CPP) results from early activation of hypothalamic GnRH secreting neurons resulting in precocious pubertal development in childhood [64,65]. Affected children present with premature development of secondary sexual characteristics, acceleration of linear growth and progressive skeletal maturation, resulting in premature epiphyseal closure and, consequently, short adult height in untreated cases [66]. CPP has remarkable female gender predominance and most cases are considered idiopathic, with normal central nervous system (CNS) magnetic resonance imaging (MRI) [64-68]. However, up to 75% of boys with CPP have a detectable central nervous system (CNS) lesion, mainly hypothalamic hamartomas [64-67]. Familial occurrence has been reported in 20-25% of CPP cases, suggesting a role for genetic factors in its pathogenesis [69,70]. Segregation analysis of these families suggested an autosomal dominant transmission with incomplete sex-dependent penetrance [69].

In 2008, the kisspeptin signaling pathway was implicated in the pathogenesis of CPP. The first heterozygous activating mutation of the KISS1R (p.R386P) was described in an adopted Brazilian girl with CPP [71]. She presented with slowly progressive thelarche from birth; accelerated growth, skeletal maturation and progression of breast development were noticed at seven years of age. She had pubertal estradiol levels and borderline-pubertal LH stimulated levels [71]. *In vitro* studies demonstrated that the R386P mutation, located in the carboxy terminal tail of the receptor, led to prolonged activation of intracellular signaling pathways in response to kisspeptin [71]. Therefore, in contrast to gain-of-function mutations in many G protein-coupled receptors, which cause constitutive receptor activation, the p.R386P mutation appeared to reduce the rate of desensitization of the mutant KISS1R at the cell surface after ligand-binding and signaling. Indeed, the p.R386P mutation appears to decrease KISS1R degradation, resulting in a net increase of the receptor on the plasma membrane [72].

Given the description of an activating mutation in *KISS1R* causing premature activation of the gonadotropic axis, *KISS1* was another obvious natural candidate gene for precocious puberty. One rare kisspeptin variant, p.P74S, was identified in one child with sporadic CPP [70]. The p.P74S mutation was identified in the heterozygous state in a boy who developed CPP at 1 yr of age with remarkably high levels of basal LH and testosterone [70]. Although the majority of boys with CPP, especially younger than 4 years old, have an underlying CNS abnormality [66-68], this boy had no CNS lesions. His mother and maternal grandmother, who had normal pubertal development, also carried the p.P74S mutation in heterozygous state, suggesting incomplete sex-dependent penetrance. After pre-incubating the mutant kisspeptin in human serum to more closely mimic *in vivo* conditions, the capacity to stimulate signal transduction was significantly greater for p.P74S compared to the wild type, suggesting that this variant might be more resistant to degradation, resulting in greater kisspeptin bioavailability [70].

The patients with *KISS1R* or *KISS1* mutations described above demonstrated adequate response to conventional treatment with GnRH agonists [70,71]. Depot GnRH agonist treatment resulted in the regression or stabilization of pubertal symptoms in the two patients

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

with activating mutations of *KISS1R* or *KISS1* genes. As expected, a decrease in the release of LH, FSH and consequently normal pre pubertal sexual steroids was achieved in these cases. In addition, the discontinuation of depot GnRH agonists treatment at the age higher than 11 years was associated with the reactivation of the reproductive axis in both cases, suggesting that the clinical and hormonal features of patients with activating mutations of the *KISS1R* and *KISS1* gene were not different from children with idiopathic or organic causes of central precocious puberty.

Although these case reports expand the genotype-phenotype correlations for the kisspeptin pathway, no other CPP cases with activating *KISS1R* or *KISS1* mutations have been reported, suggesting that these genetic abnormalities are very rare. Other cohorts have been screened, but no mutations have been identified in the *KISS1* gene [73,74]. Considering the low incidence of mutations in these genes in relation to the frequency of familial CPP, it is possible that other genes involved in GnRH regulation also will bear activating or inactivating mutations. Indeed, loss-of-function mutations affecting repressor genes of the GnRH gene might play a role in the pathogenesis of non organic CPP in the future.

Kisspeptin Expression in Organic Central Precocious Puberty

Hypothalamic hamartomas are the most common identifiable cause of CPP. Certain characteristics of anatomy and neuropeptide expression have been proposed to be associated with CPP. The expression of GnRH, GnRH receptor, TGFalpha, KISS1, KISS1R and GRM1A was investigated in hamartomas associated with or without precocious sexual development [75]. Hypothalamic hamartomas associated with CPP were larger than those not associated with CPP and were more likely to contact the infundibulum or tuber cinereum. However, the expression of *KISS1* and *KISS1R* was similar in both groups, demonstrating that expression of this signaling pathway does not differentiate between hamartomas associated with precocity vs. hamartomas that are identified in the setting of normal pubertal development [75].

Conclusions

Mutations in genes associated with IHH have been identified in approximately 30-40% of the patients with GnRH deficiency. Although mutations in *KISS1*/*KISS1R* are not a common cause of hypogonadotropism or CPP, the discovery of defects in this pathway in IHH as well as in CPP has shed some light on the mechanisms involved in GnRH secretion regulation, revealing the critical role played by the kisspeptin signaling pathway in pubertal initiation and reproductive function.

Considering the low incidence of mutations in these genes in precocious puberty so far, other genes involved in the HPG axis modulation, particularly factors upstream from, the *KISS1*/*KiSS1R* systems, might be involved in CPP pathogenesis. New methodologies, such as next generation sequencing and comparative genomic hybridization (CGH), will provide a more comprehensive assessment of genomic abnormalities, and allow new genes to be uncovered.

Acknowledgments

Dr. Seminara and some of the data referenced in this article were supported by the Eunice Kennedy Shriver NICHD/NIH through cooperative agreement [U54 HD028138] as part of the Specialized Cooperative Centers Program in Reproduction and Infertility Research, as well as R01 HD043341, and K24 HD067388.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

References

1. Crowley WF Jr. McArthur JW. Simulation of the normal menstrual cycle in Kallman's syndrome by pulsatile administration of luteinizing hormone-releasing hormone (LHRH). J Clin Endocrinol Metab. 1980; 51:173–175. [PubMed: 6247361]
2. Hoffman AR, Crowley WF Jr. Induction of puberty in men by long-term pulsatile administration of low-dose gonadotropin-releasing hormone. N Engl J Med. 1982; 307:1237–1241. [PubMed: 6813732]
3. de Roux N, Genin E, Carel JC, Matsuda F, Chaussain JL, Milgrom E. Hypogonadotropic hypogonadism due to loss of function of the KiSS1-derived peptide receptor GPR54. Proc Natl Acad Sci U S A. 2003; 100:10972–10976. [PubMed: 12944565]
4. Seminara SB, Messager S, Chatzidaki EE, Thresher RR, Acierno JS Jr. Shagoury JK, Bo-Abbas Y, Kuohung W, Schwinof KM, Hendrick AG, Zahn D, Dixon J, Kaiser UB, Slaugenhaupt SA, Gusella JF, O'Rahilly S, Carlton MB, Crowley WF Jr. Aparicio SA, Colledge WH. The GPR54 gene as a regulator of puberty. N Engl J Med. 2003; 349:1614–1627. [PubMed: 14573733]
5. Maquat LE, Carmichael GG. Quality control of mRNA function. Cell. 2001; 104:173–176. [PubMed: 11207359]
6. Frischmeyer PA, van Hoof A, O'Donnell K, Guerrerio AL, Parker R, Dietz HC. An mRNA surveillance mechanism that eliminates transcripts lacking termination codons. Science. 2002; 295:2258–2261. [PubMed: 11910109]
7. van Hoof A, Frischmeyer PA, Dietz HC, Parker R. Exosome-mediated recognition and degradation of mRNAs lacking a termination codon. Science. 2002; 295:2262–2264. [PubMed: 11910110]
8. Chan YM, Broder-Fingert S, Wong KM, Seminara SB. Kisspeptin/Gpr54-independent gonadotrophin-releasing hormone activity in Kiss1 and Gpr54 mutant mice. J Neuroendocrinol. 2009; 21:1015–1023. [PubMed: 19840236]
9. Funes S, Hedrick JA, Vassileva G, Markowitz L, Abbondanzo S, Golovko A, Yang S, Monsma FJ, Gustafson EL. The KiSS-1 receptor GPR54 is essential for the development of the murine reproductive system. Biochem Biophys Res Commun. 2003; 312:1357–1363. [PubMed: 14652023]
10. Gottsch ML, Cunningham MJ, Smith JT, Popa SM, Acohido BV, Crowley WF, Seminara S, Clifton DK, Steiner RA. A role for kisspeptins in the regulation of gonadotropin secretion in the mouse. Endocrinology. 2004; 145:4073–4077. [PubMed: 15217982]
11. Shahab M, Mastronardi C, Seminara SB, Crowley WF, Ojeda SR, Plant TM. Increased hypothalamic GPR54 signaling: a potential mechanism for initiation of puberty in primates. Proc Natl Acad Sci U S A. 2005; 102:2129–2134. [PubMed: 15684075]
12. Navarro VM, Castellano JM, Fernandez-Fernandez R, Barreiro ML, Roa J, Sanchez-Criado JE, Aguilar E, Dieguez C, Pinilla L, Tena-Sempere M. Developmental and hormonally regulated messenger ribonucleic acid expression of KiSS-1 and its putative receptor, GPR54, in rat hypothalamus and potent luteinizing hormone-releasing activity of KiSS-1 peptide. Endocrinology. 2004; 145:4565–4574. [PubMed: 15242985]
13. Smith JT, Dungan HM, Stoll EA, Gottsch ML, Braun RE, Eacker SM, Clifton DK, Steiner RA. Differential regulation of KiSS-1 mRNA expression by sex steroids in the brain of the male mouse. Endocrinology. 2005; 146:2976–2984. [PubMed: 15831567]
14. Smith JT, Cunningham MJ, Rissman EF, Clifton DK, Steiner RA. Regulation of Kiss1 gene expression in the brain of the female mouse. Endocrinology. 2005; 146:3686–3692. [PubMed: 15919741]
15. Irwig MS, Fraley GS, Smith JT, Acohido BV, Popa SM, Cunningham MJ, Gottsch ML, Clifton DK, Steiner RA. Kisspeptin activation of gonadotropin releasing hormone neurons and regulation of KiSS-1 mRNA in the male rat. Neuroendocrinology. 2004; 80:264–272. [PubMed: 15665556]
16. Shibata M, Friedman RL, Ramaswamy S, Plant TM. Evidence that down regulation of hypothalamic KiSS-1 expression is involved in the negative feedback action of testosterone to regulate luteinising hormone secretion in the adult male rhesus monkey (Macaca mulatta). J Neuroendocrinol. 2007; 19:432–438. [PubMed: 17504437]

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

17. Smith JT, Popa SM, Clifton DK, Hoffman GE, Steiner RA. Kiss1 neurons in the forebrain as central processors for generating the preovulatory luteinizing hormone surge. J Neurosci. 2006; 26:6687–6694. [PubMed: 16793876]

18. Kinoshita M, Tsukamura H, Adachi S, Matsui H, Uenoyama Y, Iwata K, Yamada S, Inoue K, Ohtaki T, Matsumoto H, Maeda K. Involvement of central metastin in the regulation of preovulatory luteinizing hormone surge and estrous cyclicity in female rats. Endocrinology. 2005; 146:4431–4436. [PubMed: 15976058]

19. Greives TJ, Mason AO, Scotti MA, Levine J, Ketterson ED, Kriegsfeld LJ, Demas GE. Environmental control of kisspeptin: implications for seasonal reproduction. Endocrinology. 2007; 148:1158–1166. [PubMed: 17185375]

20. Greives TJ, Kriegsfeld LJ, Demas GE. Exogenous kisspeptin does not alter photoperiod-induced gonadal regression in Siberian hamsters (Phodopus sungorus). Gen Comp Endocrinol. 2008; 156:552–558. [PubMed: 18405899]

21. Smith JT, Coolen LM, Kriegsfeld LJ, Sari IP, Jaafarzadehshirazi MR, Maltby M, Bateman K, Goodman RL, Tilbrook AJ, Ubuka T, Bentley GE, Clarke IJ, Lehman MN. Variation in kisspeptin and RFamide-related peptide (RFRP) expression and terminal connections to gonadotropin-releasing hormone neurons in the brain: a novel medium for seasonal breeding in the sheep. Endocrinology. 2008; 149:5770–5782. [PubMed: 18617612]

22. Greives TJ, Humber SA, Goldstein AN, Scotti MA, Demas GE, Kriegsfeld LJ. Photoperiod and testosterone interact to drive seasonal changes in kisspeptin expression in Siberian hamsters (Phodopus sungorus). J Neuroendocrinol. 2008; 20:1339–1347. [PubMed: 19094081]

23. Smith JT, Saleh SN, Clarke IJ. Seasonal and cyclical change in the luteinizing hormone response to kisspeptin in the ewe. Neuroendocrinology. 2009; 90:283–291. [PubMed: 19590160]

24. Paul MJ, Pyter LM, Freeman DA, Galang J, Prendergast BJ. Photic and nonphotic seasonal cues differentially engage hypothalamic kisspeptin and RFamide-related peptide mRNA expression in Siberian hamsters. J Neuroendocrinol. 2009; 21:1007–1014. [PubMed: 19840238]

25. Chalivoix S, Bagnolini A, Caraty A, Cognie J, Malpaux B, Dufourny L. Effects of photoperiod on Kisspeptin neuronal populations of the ewe diencephalon in connection with reproductive function. J Neuroendocrinol. 2009

26. Smith JT, Acohido BV, Clifton DK, Steiner RA. KiSS-1 neurones are direct targets for leptin in the ob/ob mouse. J Neuroendocrinol. 2006; 18:298–303. [PubMed: 16503925]

27. Castellano JM, Navarro VM, Fernandez-Fernandez R, Roa J, Vigo E, Pineda R, Dieguez C, Aguilar E, Pinilla L, Tena-Sempere M. Expression of hypothalamic KiSS-1 system and rescue of defective gonadotropic responses by kisspeptin in streptozotocin-induced diabetic male rats. Diabetes. 2006; 55:2602–2610. [PubMed: 16936210]

28. Sykiotis GP, Plummer L, Hughes VA, Au M, Durrani S, Nayak-Young S, Dwyer AA, Quinton R, Hall JE, Gusella JF, Seminara SB, Crowley WF Jr. Pitteloud N. Oligogenic basis of isolated gonadotropin-releasing hormone deficiency. Proc Natl Acad Sci U S A. 2010; 107:15140–15144. [PubMed: 20696889]

29. Bhagavath B, Xu N, Ozata M, Rosenfield RL, Bick DP, Sherins RJ, Layman LC. KAL1 mutations are not a common cause of idiopathic hypogonadotrophic hypogonadism in humans. Mol Hum Reprod. 2007; 13:165–170. [PubMed: 17213338]

30. Albuisson J, Pecheux C, Carel JC, Lacombe D, Leheup B, Lapuzina P, Bouchard P, Legius E, Matthijs G, Wasniewska M, Delpech M, Young J, Hardelin JP, Dode C. Kallmann syndrome: 14 novel mutations in KAL1 and FGFR1 (KAL2). Hum Mutat. 2005; 25:98–99. [PubMed: 15605412]

31. Bilban M, Ghaffari-Tabrizi N, Hintermann E, Bauer S, Molzer S, Zoratti C, Malli R, Sharabi A, Hiden U, Graier W, Knofler M, Andreae F, Wagner O, Quaranta V, Desoye G. Kisspeptin-10, a KiSS-1/metastin-derived decapeptide, is a physiological invasion inhibitor of primary human trophoblasts. J Cell Sci. 2004; 117:1319–1328. [PubMed: 15020672]

32. Roseweir AK, Katz AA, Millar RP. Kisspeptin-10 inhibits cell migration in vitro via a receptor-GSK3 beta-FAK feedback loop in HTR8SVneo cells. Placenta. 2012; 33:408–415. [PubMed: 22349353]

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

33. Lee JH, Welch DR. Suppression of metastasis in human breast carcinoma MDAMB-435 cells after transfection with the metastasis suppressor gene, KiSS-1. Cancer Res. 1997; 57:2384–2387. [PubMed: 9192814]
34. Miele ME, Robertson G, Lee JH, Coleman A, McGary CT, Fisher PB, Lugo TG, Welch DR. Metastasis suppressed, but tumorigenicity and local invasiveness unaffected, in the human melanoma cell line MelJuSo after introduction of human chromosomes 1 or 6. Mol Carcinog. 1996; 15:284–299. [PubMed: 8634087]
35. Lanfranco F, Gromoll J, von Eckardstein S, Herding EM, Nieschlag E, Simoni M. Role of sequence variations of the GnRH receptor and G protein-coupled receptor 54 gene in male idiopathic hypogonadotropic hypogonadism. Eur J Endocrinol. 2005; 153:845–852. [PubMed: 16322390]
36. Teles MG, Trarbach EB, Noel SD, Guerra-Junior G, Jorge A, Beneduzzi D, Bianco SD, Mukherjee A, Baptista MT, Costa EM, De Castro M, Mendonca BB, Kaiser UB, Latronico AC. A novel homozygous splice acceptor site mutation of KISS1R in two siblings with normosmic isolated hypogonadotropic hypogonadism. Eur J Endocrinol. 2010; 163:29–34. [PubMed: 20371656]
37. Tenenbaum-Rakover Y, Commenges-Ducos M, Iovane A, Aumas C, Admoni O, de Roux N. Neuroendocrine phenotype analysis in five patients with isolated hypogonadotropic hypogonadism due to a L102P inactivating mutation of GPR54. J Clin Endocrinol Metab. 2007; 92:1137–1144. [PubMed: 17164310]
38. Bo-Abbas Y, Acierno JS Jr. Shagoury JK, Crowley WF Jr. Seminara SB. Autosomal recessive idiopathic hypogonadotropic hypogonadism: genetic analysis excludes mutations in the gonadotropin-releasing hormone (GnRH) and GnRH receptor genes. J Clin Endocrinol Metab. 2003; 88:2730–2737. [PubMed: 12788881]
39. Pallais JC, Bo-Abbas Y, Pitteloud N, Crowley WF Jr. Seminara SB. Neuroendocrine, gonadal, placental, and obstetric phenotypes in patients with IHH and mutations in the G-protein coupled receptor, GPR54. Mol Cell Endocrinol. 2006; 254:255–70.
40. Nimri R, Lebenthal Y, Lazar L, Chevrier L, Phillip M, Bar M, Hernandez-Mora E, de Roux N, Gat-Yablonski G. A Novel Loss-of-Function Mutation in GPR54/KISS1R Leads to Hypogonadotropic Hypogonadism in a Highly Consanguineous Family. J Clin Endocrinol Metab. 2010
41. Semple RK, Achermann JC, Ellery J, Farooqi IS, Karet FE, Stanhope RG, O'Rahilly S, Aparicio SA. Two novel missense mutations in g protein-coupled receptor 54 in a patient with hypogonadotropic hypogonadism. J Clin Endocrinol Metab. 2005; 90:1849–1855. [PubMed: 15598687]
42. Wahab F, Quinton R, Seminara SB. The kisspeptin signaling pathway and its role in human isolated GnRH deficiency. Mol Cell Endocrinol. 2011; 346:29–36. [PubMed: 21704672]
43. Goodman RL, Lehman MN, Smith JT, Coolen LM, de Oliveira CV, Jafarzadehshirazi MR, Pereira A, Iqbal J, Caraty A, Ciofi P, Clarke IJ. Kisspeptin neurons in the arcuate nucleus of the ewe express both dynorphin A and neurokinin B. Endocrinology. 2007; 148:5752–5760. [PubMed: 17823266]
44. Rance NE. Menopause and the human hypothalamus: evidence for the role of kisspeptin/neurokinin B neurons in the regulation of estrogen negative feedback. Peptides. 2009; 30:111–122. [PubMed: 18614256]
45. Hrabovszky E, Ciofi P, Vida B, Horvath MC, Keller E, Caraty A, Bloom SR, Ghatei MA, Dhillo WS, Liposits Z, Kallo I. The kisspeptin system of the human hypothalamus: sexual dimorphism and relationship with gonadotropin-releasing hormone and neurokinin B neurons. Eur J Neurosci. 2010; 31:1984–1998. [PubMed: 20529119]
46. Topaloglu AK, Reimann F, Guclu M, Yalin AS, Kotan LD, Porter KM, Serin A, Mungan NO, Cook JR, Ozbek MN, Imamoglu S, Akalin NS, Yuksel B, O'Rahilly S, Semple RK. TAC3 and TACR3 mutations in familial hypogonadotropic hypogonadism reveal a key role for Neurokinin B in the central control of reproduction. Nat Genet. 2009; 41:354–358. [PubMed: 19079066]
47. Gianetti E, Tusset C, Noel SD, Au MG, Dwyer AA, Hughes VA, Abreu AP, Carroll J, Trarbach E, Silveira LF, Costa EM, de Mendonca BB, de Castro M, Lofrano A, Hall JE, Bolu E, Ozata M, Quinton R, Amory JK, Stewart SE, Arlt W, Cole TR, Crowley WF, Kaiser UB, Latronico AC, Seminara SB. TAC3/TACR3 Mutations Reveal Preferential Activation of Gonadotropin-Releasing

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Hormone Release by Neurokinin B in Neonatal Life Followed by Reversal in Adulthood. J Clin Endocrinol Metab.

48. Yang JJ, Caligioni CS, Chan YM, Seminara SB. Uncovering novel reproductive defects in neurokinin B receptor null mice: closing the gap between mice and men. Endocrinology. 2012; 153:1498–1508. [PubMed: 22253416]
49. Pitteloud N, Boepple PA, DeCruz S, Valkenburgh SB, Crowley WF Jr. Hayes FJ. The fertile eunuch variant of idiopathic hypogonadotropic hypogonadism: spontaneous reversal associated with a homozygous mutation in the gonadotropin-releasing hormone receptor. J Clin Endocrinol Metab. 2001; 86:2470–2475. [PubMed: 11397842]
50. Dewailly D, Boucher A, Decanter C, Lagarde JP, Counis R, Kottler ML. Spontaneous pregnancy in a patient who was homozygous for the Q106R mutation in the gonadotropin-releasing hormone receptor gene. Fertil Steril. 2002; 77:1288–1291. [PubMed: 12057744]
51. Pitteloud N, Acierno JS Jr. Meysing AU, Dwyer AA, Hayes FJ, Crowley WF Jr. Reversible kallmann syndrome, delayed puberty, and isolated anosmia occurring in a single family with a mutation in the fibroblast growth factor receptor 1 gene. J Clin Endocrinol Metab. 2005; 90:1317–1322. [PubMed: 15613419]
52. Raivio T, Falardeau J, Dwyer A, Quinton R, Hayes FJ, Hughes VA, Cole LW, Pearce SH, Lee H, Boepple P, Crowley WF Jr. Pitteloud N. Reversal of idiopathic hypogonadotropic hypogonadism. N Engl J Med. 2007; 357:863–873. [PubMed: 17761590]
53. Ribeiro RS, Vieira TC, Abucham J. Reversible Kallmann syndrome: report of the first case with a KAL1 mutation and literature review. Eur J Endocrinol. 2007; 156:285–290. [PubMed: 17322486]
54. Sinisi AA, Asci R, Bellastella G, Maione L, Esposito D, Elefante A, De Bellis A, Bellastella A, Iolascon A. Homozygous mutation in the prokineticin-receptor2 gene (Val274Asp) presenting as reversible Kallmann syndrome and persistent oligozoospermia: case report. Hum Reprod. 2008; 23:2380–2384. [PubMed: 18596028]
55. Young J, George JT, Tello JA, Francou B, Bouligand J, Guiochon-Mantel A, Brailly-Tabard S, Anderson RA, Millar RP. Kisspeptin Restores Pulsatile LH Secretion in Patients with Neurokinin B Signaling Deficiencies: Physiological, Pathophysiological and Therapeutic Implications. Neuroendocrinology. 2012
56. Chan YM, Broder-Fingert S, Paraschos S, Lapatto R, Au M, Hughes V, Bianco SD, Min L, Plummer L, Cerrato F, De Guillebon A, Wu IH, Wahab F, Dwyer A, Kirsch S, Quinton R, Cheetham T, Ozata M, Ten S, Chanoine JP, Pitteloud N, Martin KA, Schiffmann R, Van der Kamp HJ, Nader S, Hall JE, Kaiser UB, Seminara SB. GnRH-deficient phenotypes in humans and mice with heterozygous variants in KISS1/Kiss1. J Clin Endocrinol Metab. 2011; 96:E1771–1781. [PubMed: 21880801]
57. Topaloglu AK, Tello JA, Kotan LD, Ozbek MN, Yilmaz MB, Erdogan S, Gurbuz F, Temiz F, Millar RP, Yuksel B. Inactivating KISS1 mutation and hypogonadotropic hypogonadism. N Engl J Med. 2012; 366:629–635. [PubMed: 22335740]
58. d'Anglemont de Tassigny X, Fagg LA, Dixon JP, Day K, Leitch HG, Hendrick AG, Zahn D, Franceschini I, Caraty A, Carlton MB, Aparicio SA, Colledge WH. Hypogonadotropic hypogonadism in mice lacking a functional Kiss1 gene. Proc Natl Acad Sci U S A. 2007; 104:10714–10719. [PubMed: 17563351]
59. Lapatto R, Pallais JC, Zhang D, Chan YM, Mahan A, Cerrato F, Le WW, Hoffman GE, Seminara SB. Kiss1−/− mice exhibit more variable hypogonadism than Gpr54−/− mice. Endocrinology. 2007; 148:4927–4936. [PubMed: 17595229]
60. Mayer C, Boehm U. Female reproductive maturation in the absence of kisspeptin/GPR54 signaling. Nat Neurosci. 2011; 14:704–710. [PubMed: 21516099]
61. Gibson MJ, Krieger DT, Charlton HM, Zimmerman EA, Silverman AJ, Perlow MJ. Mating and pregnancy can occur in genetically hypogonadal mice with preoptic area brain grafts. Science. 1984; 225:949–951. [PubMed: 6382608]
62. Gibson MJ, Charlton HM, Perlow MJ, Zimmerman EA, Davies TF, Krieger DT. Preoptic area brain grafts in hypogonadal (hpg) female mice abolish effects of congenital hypothalamic gonadotropin-releasing hormone (GnRH) deficiency. Endocrinology. 1984; 114:1938–1940. [PubMed: 6370670]

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

63. Steiner, RA. How many kisses are required to change a frog into a princess?. Presented at the 94th Annual Meeting of the Endocrine Society; Houston TX, United States. 2012; Symposium S38-2 “New Insights into the Regulation of Puberty”
64. Palmert MR, Boepple PA. Variation in the timing of puberty: clinical spectrum and genetic investigation. J Clin Endocrinol Metab. 2001; 86:2364–2368. [PubMed: 11397824]
65. Partsch CJ, Heger S, Sippell WG. Management and outcome of central precocious puberty. Clin Endocrinol (Oxf). 2002; 56:129–148. [PubMed: 11874402]
66. Brito VN, Latronico AC, Arnhold IJ, Mendonca BB. Update on the etiology, diagnosis and therapeutic management of sexual precocity. Arq Bras Endocrinol Metabol. 2008; 52:18–31. [PubMed: 18345393]
67. Kakarla N, Bradshaw KD. Disorders of pubertal development: precocious puberty. Semin Reprod Med. 2003; 21:339–351. [PubMed: 14724767]
68. Brito VN, Latronico AC, Arnhold IJ, Lo LS, Domenice S, Albano MC, Fragoso MC, Mendonca BB. Treatment of gonadotropin dependent precocious puberty due to hypothalamic hamartoma with gonadotropin releasing hormone agonist depot. Arch Dis Child. 1999; 80:231–234. [PubMed: 10325702]
69. de Vries L, Kauschansky A, Shohat M, Phillip M. Familial central precocious puberty suggests autosomal dominant inheritance. J Clin Endocrinol Metab. 2004; 89:1794–1800. [PubMed: 15070947]
70. Silveira LG, Noel SD, Silveira-Neto AP, Abreu AP, Brito VN, Santos MG, Bianco SD, Kuohung W, Xu S, Gryngarten M, Escobar ME, Arnhold IJ, Mendonca BB, Kaiser UB, Latronico AC. Mutations of the KISS1 gene in disorders of puberty. J Clin Endocrinol Metab. 95:2276–2280. [PubMed: 20237166]
71. Teles MG, Bianco SD, Brito VN, Trarbach EB, Kuohung W, Xu S, Seminara SB, Mendonca BB, Kaiser UB, Latronico AC. A GPR54-activating mutation in a patient with central precocious puberty. N Engl J Med. 2008; 358:709–715. [PubMed: 18272894]
72. Bianco SD, Vandepas L, Correa-Medina M, Gereben B, Mukherjee A, Kuohung W, Carroll R, Teles MG, Latronico AC, Kaiser UB. KISS1R intracellular trafficking and degradation: effect of the Arg386Pro disease-associated mutation. Endocrinology. 152:1616–1626. [PubMed: 21285314]
73. Ko JM, Lee HS, Hwang JS. KISS1 gene analysis in Korean girls with central precocious puberty: a polymorphism, p.P110T, suggested to exert a protective effect. Endocr J. 57:701–709. [PubMed: 20631455]
74. Tommiska J, Sorensen K, Aksglaede L, Koivu R, Puhakka L, Juul A, Raivio T. LIN28B, LIN28A, KISS1, and KISS1R in idiopathic central precocious puberty. BMC Res Notes. 4:363. [PubMed: 21939553]
75. Chan YM, Fenoglio-Simeone KA, Paraschos S, Muhammad L, Troester MM, Ng YT, Johnsonbaugh RE, Coons SW, Prenger EC, Kerrigan JF Jr. Seminara SB. Central precocious puberty due to hypothalamic hamartomas correlates with anatomic features but not with expression of GnRH, TGFalpha, or KISS1. Horm Res Paediatr. 73:312–319. [PubMed: 20389100]