Published in final edited form as:
Mol Cell Endocrinol. 2011 October 22; 346(0): 29–36. doi:10.1016/j.mce.2011.05.043.

The Kisspeptin Signaling Pathway and its Role in Human Isolated GnRH Deficiency

Fazal Wahab[a], **Richard Quinton**[b], and **Stephanie B. Seminara**[a]

[a]Harvard Center for Reproductive Sciences and Reproductive Endocrine Unit, Department of Medicine, Massachusetts General Hospital, Boston, Massachusetts 02114

[b]Endocrinology Research Group, Institute of Human Genetics, University of Newcastle-upon-Tyne, Newcastle-upon-Tyne NE1 3BZ, United Kingdom

Abstract

Amplification of the neurosecretory activity of the GnRH system is the defining neuroendocrine even for sexual maturation. The physiological mechanisms that drive GnRH secretion at puberty have been difficult to identify but the discovery in 2003 that the G protein coupled receptor KISS1R is a key regulator of pubertal development in mice and men has ushered in a new chapter in reproductive neuroendocrinology. KISS1R is activated by endogenous peptides derived from a precursor protein, kisspeptin. Despite kisspeptin's importance in driving the reproductive cascade, relatively few patients with GnRH deficient states and mutations in the kisspeptin pathway have been described. Yet, these cases, coupled with loss-of-function mouse models, provide unique and complementary information into the biological role of this signaling system in the control of GnRH secretion. This article will examine some of the subtleties in genotype-phenotype correlations in both mice and men carrying disabling mutations in the kisspeptin pathway.

Introduction to Genetics of GnRH Deficiency

As outlined in Chapter 1 by Balasubramanian and Crowley, in the last two decades, several genes and pathways which govern GnRH ontogeny have been discovered by studying humans with GnRH deficiency, a condition characterized by abnormal pubertal development and low gonadotropins and sex steroids. These pathways include genes whose protein products influence GnRH neuronal migration to the hypothalamus such as *KAL1* (1-2), *FGFR1* (3-4), *FGF8* (5), *NELF* (6-7), PROK2 (8-9); GnRH synthesis (*GNRH1*)(10-12); GnRH responsiveness at the pituitary (*GNRHR*)(13-15); and biologic functions yet to be understood (*CHD7*)(16) and possibly *WDR11* (17). In 2003, homozygosity mapping and candidate gene analysis of two large consanguineous pedigrees led to the identification of loss of function mutations in *KISS1R* (a G protein coupled receptor with homology to the galanin receptor family) by two investigative groups (18-19). In parallel, the *Kiss1r*$^{-/-}$ mouse demonstrated a phenotype that reinforced the human GnRH deficient state, demonstrating that the function of KISS1R/Kiss1r is conserved across mammalian species (19-20). Just prior to these human genetic discoveries, the ligand for KISS1R (also known as GPR54), was discovered to be kisspeptin, an RF amide using both biological and bioinformatic approaches (21-23). Soon thereafter, 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

Corresponding author: Stephanie B. Seminara, MD, FACP, Associate Professor of Medicine, Harvard Medical School, Harvard Reproductive Endocrine Sciences Center and Reproductive Endocrine Unit of the Department of Medicine, Massachusetts General Hospital, Fruit Street, Boston MA 02114, Tel: 617 724 8304, Fax: 617 726 5357, sseminara@partners.org.

secretion. This article will examine genotype phenotype correlations within the kisspeptin pathway in both mice and men.

The Role of the Kisspeptin Signaling Pathway in Reproduction

Whereas loss of function mutations in *Kiss1r* and *KISS1R* causes hypogonadotropic hypogonadism in mice and men (18-19), *Kiss1r*$^{-/-}$ mice permitted an examination of the hypothalamic content of GnRH which proved to be normal, leading to the hypothesis that kisspeptin does not affect GnRH neuronal fate specification, migration, or migration nor its biosynthesis but rather, its release (19). Soon thereafter, kisspeptin administration, either centrally or peripherally, was shown to selectively stimulate the secretion of GnRH and, in turn, LH and FSH. Moreover, this peptide proved to be the most potent GnRH releasing hormone that had ever been studied (24). Vaginal opening in juvenile mice treated with kisspeptin (25) is advanced and kisspeptin administration can rescue the delayed puberty caused by leptin deficiency (26). Kisspeptin expression in the hypothalamus of rodents and non-human primates increases at the time of sexual maturation (27-28), an important finding since kisspeptin is thought to mediate sex steroid feedback (27, 29-33), estrous cycle regulation (33-34), seasonal breeding (35-41), and to convey information about the energy status of the organism (42-43). Thus, it was evident early in the kisspeptin story that the kisspeptin signaling system plays a central role in integrating numerous signals previously demonstrated to modulate GnRH neuronal secretion. Kisspeptin is thus a key gatekeeper for the activation of the GnRH axis.

Summary of KISS1R Mutations

Curiously, relatively few loss-of-function mutations in *KISS1R* have been reported. Biallelic mutations include a homozygous 155 base pair deletion (18), a homozygous frameshift (1001_1002insC) (44), a homozygous splice acceptor site mutation (45), homozygous L102P (46), L148S (19, 47-48) and F272S missense mutations (49), and compound heterozyous mutations R331X/X399R (19, 48) and R297L/C223R (50) (cf Figure 1). As can easily be appreciated from this listing, mutations in this G protein coupled receptor are variable in type (large deletion, frameshift, splice site, nonsense, nonstop, and missense). Thus single nucleotide changes leading to putative missense changes occur throughout the receptor and are not clustered into a single hot spot.

While biallelic mutations in *KISS1R* are clearly associated with hypogonadotropism, it is important to recall that GnRH deficiency can be partial or complete (by history, physical examination, and baseline neuroendocrine and gonadal testing) (51), permanent or reversible (52), and congenital or adult in onset (53). In addition, GnRH deficient patients can be responsive or unresponsive to exogenous GnRH during single bolus stimulation testing but typically respond to prolonged pulsatile GnRH therapy in most cases (54). Therefore, the more complete the baseline neuroendocrine profile and the subsequent charting of responsiveness to long term GnRH administration in patients carrying variants in *KISS1R*, the greater the pathophysiologic insights that can be obtained by study of their genotype/phenotype correlations.

Neuroendocrine Phenotypes in Patients Carrying Biallelic Complete Loss of Function Mutations in KISS1R: Evidence for Persistent GnRH Secretion

Typically, patients carrying biallelic complete loss-of-function mutations serve as markers of the most extreme phenotype that can be associated with loss of a particular gene. However, complete loss of kisspeptin signaling is not always associated with complete

GnRH deficiency. The following three cases illustrate this concept (cf. Table 1 for phenotypic details).

Five affected siblings from a large consanguineous family with GnRH deficiency were found to carry a homozygous 155 base pair deletion in *KISS1R* (18). The only affected female presented with some partial breast development and one episode of uterine bleeding. Although her sexual maturation was clearly abnormal, her breast development and uterine bleeding suggest her endogenous estradiol levels were above pre-pubertal values. As part of her baseline diagnostic evaluation, she received 100 μg of GnRH IV which caused her LH to rise from 2.0 to a peak of 11.8 mIU/mL and her FSH from 3.4 to 6.4 mIU/mL, suggesting some gonadotrope responsiveness most likely due to some prior pituitary exposure to GnRH.

An African American patient male carrying the compound heterozygous mutations R331X/X399R presented at age 17 10/12 y for pubertal delay and decreased libido (19, 48). However, despite physical examination correlates of severe androgen deficiency from the 'minipuberty' of infancy (microphallus and pre-pubertal sized testes), his gonadotropin levels rose robustly after a GnRH challenge. During frequent blood sampling (q 10 minutes), the patient demonstrated 9 low amplitude LH pulses (19). Eight months after long-term therapy with exogenous pulsatile GnRH (q 2h SC), the R331X/X399R patient demonstrated a left shifted dose-response curve to exogenous GnRH compared to 6 normosmic GnRH deficient men undergoing an identical protocol. Collectively, his responses to the single bolus GnRH test, his baseline LH pulses, and his dose-response study all suggest the presence of some degree of low-level, endogenous pulsatile GnRH secretion.

These observations were echoed in a female proband of Syrian descent who carries homozygous L102P mutations in the first extracellular loop of KISS1R (46). This patient also showed a robust LH response (peak value approximately 32 IU/L) in response to a 100μg GnRH stimulation test at baseline assessment. Although only 6 hours of frequent blood sampling was performed, she manifested low amplitude LH pulses occurring approximately once per hour. Another Middle Eastern proband from a separate family, a male carrying homozygous L102P (46), received 6 GnRH stimulation tests from age 12.5 to 21.6 years. Over time, in response to the exogenous GnRH, this patient's LH/FSH ratio changed from less than one (ages 12.5 to 18 y) to greater than one (ages 19.8 to 21.6 years). This maturational switch in gonadotropin ratio values, directly the same as occurs in boys undergoing a normal puberty, could also be explained by the presence of faint but present endogenous GnRH secretion and resultant pituitary priming.

Therefore, the phenotypes of patients carrying the homozygous 155 base pair deletion, compound heterozygous R331X/X399R, and homozygous L102P collectively suggest that evidence for some endogenous GnRH secretion can exist in the face of loss of function kisspeptin receptor mutations. This residual GnRH secretion might be caused by persistent kisspeptin signaling if the *KISS1R* mutations were not completely disabling. However, all 3 sets of mutations listed above have dramatic effects on kisspeptin signaling when tested *in vitro*. The 155 base pair deletion removes the splicing acceptor site of the intron 4-5 junction and part of exon 5 (18). Even if the mutant alleles containing this deletion were transcribed, they would create a receptor protein lacking transmembrane domains 6 and 7 that would presumably be unable to stimulate second messenger signaling. The R331X and X399R mutations create transcripts that are hypothesized to undergo degradation by nonsense- and nonstop-mediated decay mechanisms (19). Even if these transcripts escaped their respective decay processes, *in vitro* studies demonstrated that the mutant receptors created by each of these transcripts functioned poorly. Finally, the L102P mutation is a missense mutation as opposed to a frameshift, nonsense or nonstop. However, L102P was found to completely

abolish inositol phosphate accumulation *in vitro* (46). Therefore, despite apparently completely disabling mutations at least by this bioassay, three individuals with severe biallelic *KISS1R* mutations demonstrated evidence for persistent, albeit abnormal, GnRH secretion as attested to by their partial phenotypes. So either these mutations retain some degree of signaling that was not evident in the *in vitro* assay systems used or there is some 'kisspeptin-independent' GnRH. This theme of kisspeptin-independent GnRH signaling is echoed in *Kiss1*$^{-/-}$ and *Kiss1r*$^{-/-}$ mice, described later in this article.

A Case of Genotype/Phenotype Discordance?

A challenging case is that of a boy of who presented with micropenis and cryptorchidism who was found to harbor C223R/R297L variants in *KISS1R* (50). Neuroendocrine data obtained from the first ten years of life suggested that this patient has severe GnRH deficiency with defects at multiple levels of the hypothalamic-pituitary-gonadal axis. However, this patient did not have two severe mutations. Although the C223R mutation was found to impair signaling profoundly, the R297L variant resulted in a much milder impairment of receptor activity, evidenced by effect on real-time measurements of calcium flux.

So how does one resolve this patient's severe neuroendocrine phenotype with the relatively "mild" genotype evidenced by functional studies of his mutations? That this evaluation was performed before the expected time of puberty may be relevant. The principal modulators of GnRH secretion during the neonatal window may not be identical to those that predominate during adolescent puberty, raising the possibility that with increasing age, this patient might indeed come to display some of the neuroendocrine patterns of the previously-described patients. For example, although his response to the GnRH stimulation test at 10 y was poor, it remains possible that his response to GnRH might improve if he were tested through his teens or early twenties. This patient's poor response to exogenous hCG is notable since other patients bearing *KISS1R* mutations have been responsive to exogenous GnRH/gonadotropins (see below), but may just reflect a negative impact of delayed orchidopexy on testicular function. Although the patient's R297L mutation was not completely disabling in the calcium assays employed, it cannot be excluded that other functional assays looking at, for instance, synthesis, expression at the plasma membrane, and ligand binding, might reveal more deleterious effects. Finally, it is possible that this patient might carry mutations in other genes for GnRH deficiency so that his phenotype is a composite of multiple genetic defects (55).

Some phenotypic aspects of the C223R/R297L case are echoed in a family carrying a homozygous F272S mutation which almost completely inhibited kisspeptin-induced receptor signaling *in vitro* (49). F272S males had cryptorchidism and microphallus; all demonstrated blunted responses to GnRH stimulation during early infancy, again suggesting that KISS1R is required for normal functioning of the hypothalamic-pituitary-gonadal axis in infancy. (Notably, individual B3 also had a bifid scrotum and mild chordee.) Interestingly, short and prolonged hCG stimulation tests performed in infancy, childhood, adolescence and early adulthood failed to show a normal testosterone response except in one individual.

Neuroendocrine Phenotypes and Variable Expressivity

KISS1R mutations can also demonstrate variable expressivity within a family. Although four brothers with GnRH deficiency and a homozygous 155 base pair deletion in *KISS1R* demonstrated a severe phenotype, a sister bearing the same homozygous mutations had a less severe phenotype with partial breast development and one episode of uterine bleeding (17). Although all the brothers exhibited a blunted response to GnRH, the affected sister had

a peak LH of 11.8 and FSH of 6.4 units/mL. In another family with a homozygous L102P mutation, a GnRH challenge resulted in a robust LH increase in one affected female (peak approximately 8.5 IU/L) but an affected sister had practically no response. Causes of this variable expressivity are not well understood. Additional genetic inputs (oligogenicity) could account for this phenotypic heterogeneity (53). In contrast to the 155 base pair deletion pedigree, all males in the F272S family exhibited the same clinical features from infancy to adulthood, including undescended testes, microphallus, and absence of pubertal development (49). Therefore, variable expressivity is not uniformly observed within families

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* 1001_1002insC mutation responded to exogenous pulsatile GnRH, normalizing testosterone levels and inducing spermatogenesis (44). Even though his semen analysis showed oligoasthenozoospermia, pregnancy, albeit achieved with assisted reproduction, was possible. The male proband harboring R331X/X399R also received pulsatile GnRH and experienced steady increases in testicular volume and the appearance of normal spermatogenesis (48). After approximately 1 ½ years of pulsatile GnRH therapy, with a testicular volume of 12 cc bilaterally and sperm count of 7 M/mL, his partner conceived. A homozygous L148S female had 1) intact responses to exogenous GnRH and gonadotropins, 2) multiple conceptions using the aforementioned therapies as well as IVF, 3) two uncomplicated pregnancies of healthy children, 4) spontaneous initiation of uterine contractions, and 5) lactation for several months post-partum (48). While this data are consistent with an intact ovarian response, no details are available regarding the quality of her follicular response, her ability to ovulate spontaneously, and need for luteal phase support. However, in general, mutations in *KISS1R* do not appear to preclude steroidogenesis and gametogenesis.

Neuroendocrine Phenotypes in Patients Carrying Monoallelic Loss of Function Mutations in KISS1R

The contribution of heterozygous mutations in one or more genes (i.e. oligogenicity) is being increasingly recognized in genes for GnRH deficiency (53). For example, the gene encoding the prokineticin receptor, *PROKR2*, is a rich source of mutations in GnRH deficiency, the overwhelming majority of which are heterozygous (monoallelic), as opposed to biallelic variants (56-57). For *KISS1R*, not only are homozygous and compound heterozygous mutations rare, but the number of individuals carrying monoallelic variants is also small.

Previously reported heterozygous, non-synonomous variants in patients' coding sequence of *KISS1R* include L364H (50)(42), A189T (45, 58) and H360L (58). Screening a cohort of 746 probands with GnRH deficient individuals (Kallmann syndrome and normosmic hypogonadotropic hypogonadism) has revealed additional non-synonomous variants including L164F, A194D, C389X, and c.739-7delC (located just before the start of exon 5). These changes were not identified in 324 healthy controls, dbSNP (http://www.ncbi.nlm.nih.gov/projects/SNP/), and the 1000 Genome Project (http://www.1000genomes.org/). Software programs used to assess the functional significance of variants included PolyPhen (59), Mutation Taster (60), PMUT (61). All three programs suggested that L164F is benign but A194D was predicted by both PMUT and Mutation Taster to be damaging. C398X could only be evaluated by Polyphen which predicted that it was “possibly damaging,” although conventionally, premture termination codons are pathologic. For c.739-7delC, Human Splice Finder (62), suggested that the splice acceptor site may

remain the same, or it could be shifted 6 bp in the 3′ direction, deleting the first 6 bp's of exon 5. Mutation Taster predicted that even if these two amino acids were to be deleted, the change was still benign. Therefore, while not all of the newly identified heterozygote variants appear to be pathologic, others have a high likelihood of being damaging (C389X and A194D). Although detailed phenotyping information is not yet available on the patients carrying the C389X and A194D heterozygous variants, the information that is available suggests that both individuals have severe GnRH deficiency. The C389X mutation was identified in a Pakistani female who presented with primary amenorrhea, and the A195D variant was identified in a male patient with Kallmann syndrome. While the precise contribution of these heterozygous variants to the GnRH deficient phenotype is unclear, the occurrence of heterozygous mutations in genes for GnRH deficiency is being increasingly recognized—for example, the overwhelming majority of mutations in the gene encoding the prokineticin receptor, *PROKR2*, are heterozygous (56-57). Although these patients carrying C389X and A194D may also have an as-yet undiscovered genetic mutations in non-coding regions of KISS1R, it is also possible that the previous traditional monogenic status of *KISS1R* genetics may need to be supplanted by an oligogenic framework, as has been demonstrated for many other genes for GnRH deficiency (9, 63).

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

In general, *Kiss1*$^{-/-}$ and *Kiss1r*$^{-/-}$ mice phenocopy humans bearing *KISS1R* mutations. Both *Kiss1*$^{-/-}$ and *Kiss1r*$^{-/-}$ mice have abnormal sexual maturation, with small gonads, low gonadotropins, and abnormal gametogenesis (19-20, 64-65). Migration of GnRH neurons into the hypothalamus is normal in *Kiss1*$^{-/-}$ animals along with appropriate axonal connections to the median eminence and total GnRH content (64). Both *Kiss1*$^{-/-}$ and *Kiss1r*$^{-/-}$ mice are infertile as pregnancies do not occur when either mouse is housed with WT mice of proven fertility (65).

However, *Kiss1*$^{-/-}$ animals exhibit considerable phenotypic variability that is reminiscent of their human counterparts. *Kiss1*$^{-/-}$ female mice with small vaginal openings, decreased ovarian size, and scant folliculogenesis respond with the largest increments in LH and FSH to exogenous kisspeptin whereas *Kiss1*$^{-/-}$ mice with larger gonadal weights, larger vaginal openings, and persistent vaginal cornification on their smears have a less robust gonadotropin response (65). *Kiss1*$^{-/-}$ males also have a more modest phenotype than their *Kiss1r*$^{-/-}$ counterparts, although although some animals of both lines have sperm counts approaching those of WT animals (64-65).

Despite their infertility, *Kiss1*$^{-/-}$ female mice can develop follicles up to the pre-ovulatory level, although no spontaneous ovulations are observed (66). 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 completely disrupts the estrus exhibited by *Kiss1*$^{-/-}$ and *Kiss1r*$^{-/-}$ female mice, thus demonstrating that this estrus is due to GnRH activity. Although frequent blood sampling studies have not been performed in either wild type, *Kiss1*$^{-/-}$, or *Kiss1r*$^{-/-}$ mice, 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.

Understanding the contribution of other pathways to the regulation of GnRH neurons will undoubtedly be enhanced by future genetic discoveries.

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

The kisspeptin pathway stands in unique contrast to all the other pathways involved in GnRH deficiency as it harbors gain of function mutations in patients with central precocious puberty (CPP), a contrarian phenotype to GnRH deficiency. The first patient reported to carry a gain of function mutation was an 8 year old adopted Brazilian female who experienced slowly progressive premature breast development since birth (67). A rare sequence variant was detected in the C terminal tail of KISS1R (R386P). When tested, R386 mutant receptors did not increase constitutive activity of KISS1R, its binding capacity, or responsiveness to kisspeptin. However, the decline in inositol phosphate accumulation was slower in cells transfected with the R386P mutant receptor compared with WT, and the phosphorylation of extracellular signal-regulated kinase was prolonged, indicating a significant reduction in the rate of desensitization of the mutant KISS1R. Recent studies have demonstrated that R386P prolongs kisspeptin responsiveness by decreasing the degradation of its receptor (68). Thus, this case stands as a model of nonconstitutive KISS1R activation associated with precocious pubertal development.

Recently two *KISS1* mutations (P74S and H90D) were identified in three unrelated children with idiopathic CPP. The P74S mutation was identified in a boy with pubertal development beginning at 12 months (69). At 17 months, his penis was 8 × 2 cm, with testes of 2.3 × 1.5 cm (right) and 2.6 × 1.2 cm (left). His bone age was advanced to 3 years. His basal FSH was 8.3 U/L, and his basal LH was 11.5 U/L which rose after GnRH stimulation to 47.2 U/L. His testosterone was 600 ng/dL and a brain MRI was normal. He was successfully treated with a GnRH analog for 9 years. The H90D variants were identified in two girls who underwent puberty at 6 and 5.5 years of age. Both had advanced bone age and pubertal levels of estradiol and basal LH. *In vitro*, the P74S and H90D mutants stimulated IP production to a similar degree. After incubation in 50% human serum for 2 h, the dose-response curves of both the WT and P74S mutant shifted to the right; the P74S had less of a rightward shift that the WT suggesting that this variant may be more resistant to degradation. Because the P74S is located in a PEST sequence, a region associated with rapid degradation of proteins, a potential gain of function mutation might result in increased protein availability due to decreased degradation. Although the H90D variant did not show resistance to degradation, the *in vitro* studies of the P74S mutant kisspeptin suggest that it might play a role in the patient's precocious puberty phenotype.

Phenotypes Outside the Reproductive Cascade

Metabolic stress is well known to affect reproductive function and the physiologic basis for the connection between energy balance and reproduction is an area of intense investigation. It has been hypothesized that kisspeptin neurons may be sensitive to the energy reserve of the organism and may transmit information regarding metabolic status to the rest of the gonadotropic axis. This hypothesis originally emerged when it was demonstrated that rats subjected to a 3 day fast demonstrate a significant drop of Kiss1 mRNA in the hypothalamus (26). Similarly, rats in whom uncontrolled diabetes has been induced by streptozotocin develop hypogonadotropism and suppression of hypothalamic *Kiss1* mRNA (43). Links between leptin, a major indicator of energy status, and kisspeptin were first demonstrated when mice with congenital lack of leptin were shown to have significant reduction of *Kiss1* mRNA in the arcuate nucleus (42). Despite the evidence suggesting that kisspeptin neurons might mediate leptin's effects on the reproductive cascade, there are additional, kisspeptin-independent pathways that may convey leptin's effects on the reproductive axis, such as those localized to the ventral pre-mammillary nucleus (70). While the molecular mechanism underlying leptin's regulation of kisspeptin are largely unknown, recent data suggest that the

mammalian target of rapamycin (mTOR) and is down-stream effectors may be a conduit from leptin to *Kiss1* gene expression (71). As increasing number of patients with *KISS1R* mutations are identified, more provocative phenotyping studies will be required to determine if there are abnormalities in the integration of metabolic status and reproductive function

Summary

Although mutations in *KISS1R* are not a common cause of hypogonadotropism, they reveal the critical role played by the kisspeptin signaling pathway in pubertal initiation and reproductive function.

Acknowledgments

This research was supported by the Eunice Kennedy Shriver NICHD/NIH through cooperative agreement [U54 HD28138-17] as part of the Specialized Cooperative Centers Program in Reproduction and Infertility Research and R01 HD43341-09.

References

1. Franco B, Guioli S, Pragliola A, Incerti B, Bardoni B, Tonlorenzi R, Carrozzo R, Maestrini E, Pieretti M, Taillon-Miller P, et al. A gene deleted in Kallmann's syndrome shares homology with neural cell adhesion and axonal path-finding molecules. Nature. 1991; 353:529–536. [PubMed: 1922361]
2. Legouis R, Hardelin JP, Levilliers J, Claverie JM, Compain S, Wunderle V, Millasseau P, Le Paslier D, Cohen D, Caterina D, et al. The candidate gene for the X-linked Kallmann syndrome encodes a protein related to adhesion molecules. Cell. 1991; 67:423–435. [PubMed: 1913827]
3. Dode C, Levilliers J, Dupont JM, De Paepe A, Le Du N, Soussi-Yanicostas N, Coimbra RS, Delmaghani S, Compain-Nouaille S, Baverel F, Pecheux C, Le Tessier D, Cruaud C, Delpech M, Speleman F, Vermeulen S, Amalfitano A, Bachelot Y, Bouchard P, Cabrol S, Carel JC, Delemarre-van de Waal H, Goulet-Salmon B, Kottler ML, Richard O, Sanchez-Franco F, Saura R, Young J, Petit C, Hardelin JP. Loss-of-function mutations in FGFR1 cause autosomal dominant Kallmann syndrome. Nat Genet. 2003; 33:463–465. [PubMed: 12627230]
4. Pitteloud N, Acierno JS Jr, Meysing A, Eliseenkova AV, Ma J, Ibrahimi OA, Metzger DL, Hayes FJ, Dwyer AA, Hughes VA, Yialamas M, Hall JE, Grant E, Mohammadi M, Crowley WF Jr. Mutations in fibroblast growth factor receptor 1 cause both Kallmann syndrome and normosmic idiopathic hypogonadotropic hypogonadism. Proc Natl Acad Sci U S A. 2006; 103:6281–6286. [PubMed: 16606836]
5. Falardeau J, Chung WC, Beenken A, Raivio T, Plummer L, Sidis Y, Jacobson-Dickman EE, Eliseenkova AV, Ma J, Dwyer A, Quinton R, Na S, Hall JE, Huot C, Alois N, Pearce SH, Cole LW, Hughes V, Mohammadi M, Tsai P, Pitteloud N. Decreased FGF8 signaling causes deficiency of gonadotropin-releasing hormone in humans and mice. J Clin Invest. 2008; 118:2822–2831. [PubMed: 18596921]
6. Kramer PR, Wray S. Novel gene expressed in nasal region influences outgrowth of olfactory axons and migration of luteinizing hormone-releasing hormone (LHRH) neurons. Genes Dev. 2000; 14:1824–1834. [PubMed: 10898796]
7. Miura K, Acierno JS Jr, Seminara SB. Characterization of the human nasal embryonic LHRH factor gene, NELF, and a mutation screening among 65 patients with idiopathic hypogonadotropic hypogonadism (IHH). J Hum Genet. 2004; 49:265–268. [PubMed: 15362570]
8. Matsumoto S, Yamazaki C, Masumoto KH, Nagano M, Naito M, Soga T, Hiyama H, Matsumoto M, Takasaki J, Kamohara M, Matsuo A, Ishii H, Kobori M, Katoh M, Matsushime H, Furuichi K, Shigeyoshi Y. Abnormal development of the olfactory bulb and reproductive system in mice lacking prokineticin receptor PKR2. Proc Natl Acad Sci U S A. 2006; 103:4140–4145. [PubMed: 16537498]
9. Dode C, Teixeira L, Levilliers J, Fouveaut C, Bouchard P, Kottler ML, Lespinasse J, Lienhardt-Roussie A, Mathieu M, Moerman A, Morgan G, Murat A, Toublanc JE, Wolczynski S, Delpech M,

Petit C, Young J, Hardelin JP. Kallmann syndrome: mutations in the genes encoding prokineticin-2 and prokineticin receptor-2. PLoS Genet. 2006; 2:e175. [PubMed: 17054399]

10. Mason AJ, Pitts SL, Nikolics K, Szonyi E, Wilcox JN, Seeburg PH, Stewart TA. The hypogonadal mouse: reproductive functions restored by gene therapy. Science. 1986; 234:1372–1378. [PubMed: 3097822]
11. Bouligand J, Ghervan C, Tello JA, Brailly-Tabard S, Salenave S, Chanson P, Lombes M, Millar RP, Guiochon-Mantel A, Young J. Isolated familial hypogonadotropic hypogonadism and a GNRH1 mutation. N Engl J Med. 2009; 360:2742–2748. [PubMed: 19535795]
12. Chan YM, de Guillebon A, Lang-Muritano M, Plummer L, Cerrato F, Tsiaras S, Gaspert A, Lavoie HB, Wu CH, Crowley WF Jr, Amory JK, Pitteloud N, Seminara SB. GNRH1 mutations in patients with idiopathic hypogonadotropic hypogonadism. Proc Natl Acad Sci U S A. 2009; 106:11703–11708. [PubMed: 19567835]
13. de Roux N, Young J, Misrahi M, Genet R, Chanson P, Schaison G, Milgrom E. A family with hypogonadotropic hypogonadism and mutations in the gonadotropin-releasing hormone receptor. N Engl J Med. 1997; 337:1597–1602. [PubMed: 9371856]
14. Layman LC, Cohen DP, Jin M, Xie J, Li Z, Reindollar RH, Bolbolan S, Bick DP, Sherins RR, Duck LW, Musgrove LC, Sellers JC, Neill JD. Mutations in gonadotropin-releasing hormone receptor gene cause hypogonadotropic hypogonadism. Nat Genet. 1998; 18:14–15. [PubMed: 9425890]
15. Pask AJ, Kanasaki H, Kaiser UB, Conn PM, Janovick JA, Stockton DW, Hess DL, Justice MJ, Behringer RR. A novel mouse model of hypogonadotrophic hypogonadism: N-ethyl-N-nitrosourea-induced gonadotropin-releasing hormone receptor gene mutation. Mol Endocrinol. 2005; 19:972–981. [PubMed: 15625238]
16. Kim HG, Kurth I, Lan F, Meliciani I, Wenzel W, Eom SH, Kang GB, Rosenberger G, Tekin M, Ozata M, Bick DP, Sherins RJ, Walker SL, Shi Y, Gusella JF, Layman LC. Mutations in CHD7, encoding a chromatin-remodeling protein, cause idiopathic hypogonadotropic hypogonadism and Kallmann syndrome. Am J Hum Genet. 2008; 83:511–519. [PubMed: 18834967]
17. Kim HG, Ahn JW, Kurth I, Ullmann R, Kim HT, Kulharya A, Ha KS, Itokawa Y, Meliciani I, Wenzel W, Lee D, Rosenberger G, Ozata M, Bick DP, Sherins RJ, Nagase T, Tekin M, Kim SH, Kim CH, Ropers HH, Gusella JF, Kalscheuer V, Choi CY, Layman LC. WDR11, a WD protein that interacts with transcription factor EMX1, is mutated in idiopathic hypogonadotropic hypogonadism and Kallmann syndrome. Am J Hum Genet. 2010; 87:465–479. [PubMed: 20887964]
18. 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]
19. 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]
20. 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]
21. Muir AI, Chamberlain L, Elshourbagy NA, Michalovich D, Moore DJ, Calamari A, Szekeres PG, Sarau HM, Chambers JK, Murdock P, Steplewski K, Shabon U, Miller JE, Middleton SE, Darker JG, Larminie CG, Wilson S, Bergsma DJ, Emson P, Faull R, Philpott KL, Harrison DC. AXOR12, a novel human G protein-coupled receptor, activated by the peptide KiSS-1. J Biol Chem. 2001; 276:28969–28975. [PubMed: 11387329]
22. Kotani M, Detheux M, Vandenbogaerde A, Communi D, Vanderwinden JM, Le Poul E, Brezillon S, Tyldesley R, Suarez-Huerta N, Vandeput F, Blanpain C, Schiffmann SN, Vassart G, Parmentier M. The metastasis suppressor gene KiSS-1 encodes kisspeptins, the natural ligands of the orphan G protein-coupled receptor GPR54. J Biol Chem. 2001; 276:34631–34636. [PubMed: 11457843]
23. Ohtaki T, Shintani Y, Honda S, Matsumoto H, Hori A, Kanehashi K, Terao Y, Kumano S, Takatsu Y, Masuda Y, Ishibashi Y, Watanabe T, Asada M, Yamada T, Suenaga M, Kitada C, Usuki S,

Kurokawa T, Onda H, Nishimura O, Fujino M. Metastasis suppressor gene KiSS-1 encodes peptide ligand of a G-protein-coupled receptor. Nature. 2001; 411:613–617. [PubMed: 11385580]

24. 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]
25. Navarro VM, Fernandez-Fernandez R, Castellano JM, Roa J, Mayen A, Barreiro ML, Gaytan F, Aguilar E, Pinilla L, Dieguez C, Tena-Sempere M. Advanced vaginal opening and precocious activation of the reproductive axis by KiSS-1 peptide, the endogenous ligand of GPR54. J Physiol. 2004; 561:379–386. [PubMed: 15486019]
26. Castellano JM, Navarro VM, Fernandez-Fernandez R, Nogueiras R, Tovar S, Roa J, Vazquez MJ, Vigo E, Casanueva FF, Aguilar E, Pinilla L, Dieguez C, Tena-Sempere M. Changes in hypothalamic KiSS-1 system and restoration of pubertal activation of the reproductive axis by kisspeptin in undernutrition. Endocrinology. 2005; 146:3917–3925. [PubMed: 15932928]
27. 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]
28. 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]
29. 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]
30. 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]
31. 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]
32. 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]
33. 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]
34. 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]
35. 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]
36. 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]
37. 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]
38. 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]

39. 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]
40. 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]
41. 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
42. 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]
43. 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]
44. 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]
45. 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]
46. 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]
47. 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]
48. 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; 254-255:70–77. [PubMed: 16757106]
49. 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
50. 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]
51. Pitteloud N, Hayes FJ, Boepple PA, DeCruz S, Seminara SB, MacLaughlin DT, Crowley WF Jr. The role of prior pubertal development, biochemical markers of testicular maturation, and genetics in elucidating the phenotypic heterogeneity of idiopathic hypogonadotropic hypogonadism. J Clin Endocrinol Metab. 2002; 87:152–160. [PubMed: 11788640]
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. Nachtigall LB, Boepple PA, Pralong FP, Crowley WF Jr. Adult-onset idiopathic hypogonadotropic hypogonadism--a treatable form of male infertility. N Engl J Med. 1997; 336:410–415. [PubMed: 9010147]
54. Sykiotis GP, Hoang XH, Avbelj M, Hayes FJ, Thambundit A, Dwyer A, Au M, Plummer L, Crowley WF Jr, Pitteloud N. Congenital idiopathic hypogonadotropic hypogonadism: evidence of defects in the hypothalamus, pituitary, and testes. J Clin Endocrinol Metab. 2010; 95:3019–3027. [PubMed: 20382682]

55. 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]
56. Cole LW, Sidis Y, Zhang C, Quinton R, Plummer L, Pignatelli D, Hughes VA, Dwyer AA, Raivio T, Hayes FJ, Seminara SB, Huot C, Alos N, Speiser P, Takeshita A, Van Vliet G, Pearce S, Crowley WF Jr, Zhou QY, Pitteloud N. Mutations in prokineticin 2 and prokineticin receptor 2 genes in human gonadotrophin-releasing hormone deficiency: molecular genetics and clinical spectrum. J Clin Endocrinol Metab. 2008; 93:3551–3559. [PubMed: 18559922]
57. Sarfati J, Guiochon-Mantel A, Rondard P, Arnulf I, Garcia-Pinero A, Wolczynski S, Brailly-Tabard S, Bidet M, Ramos-Arroyo M, Mathieu M, Lienhardt-Roussie A, Morgan G, Turki Z, Bremont C, Lespinasse J, Du Boullay H, Chabbert-Buffet N, Jacquemont S, Reach G, De Talence N, Tonella P, Conrad B, Despert F, Delobel B, Brue T, Bouvattier C, Cabrol S, Pugeat M, Murat A, Bouchard P, Hardelin JP, Dode C, Young J. A comparative phenotypic study of kallmann syndrome patients carrying monoallelic and biallelic mutations in the prokineticin 2 or prokineticin receptor 2 genes. J Clin Endocrinol Metab. 2010; 95:659–669. [PubMed: 20022991]
58. Cerrato F, Shagoury J, Kralickova M, Dwyer A, Falardeau J, Ozata M, Van Vliet G, Bouloux P, Hall JE, Hayes FJ, Pitteloud N, Martin KA, Welt C, Seminara SB. Coding sequence analysis of GNRHR and GPR54 in patients with congenital and adult-onset forms of hypogonadotropic hypogonadism. Eur J Endocrinol. 2006; 155(1):S3–S10. [PubMed: 17074994]
59. Ramensky V, Bork P, Sunyaev S. Human non-synonymous SNPs: server and survey. Nucleic Acids Res. 2002; 30:3894–3900. [PubMed: 12202775]
60. Schwarz JM, Rodelsperger C, Schuelke M, Seelow D. MutationTaster evaluates disease-causing potential of sequence alterations. Nat Methods. 2010; 7:575–576. [PubMed: 20676075]
61. Ferrer-Costa C, Gelpi JL, Zamakola L, Parraga I, de la Cruz X, Orozco M. PMUT: a web-based tool for the annotation of pathological mutations on proteins. Bioinformatics. 2005; 21:3176–3178. [PubMed: 15879453]
62. Desmet FO, Hamroun D, Lalande M, Collod-Beroud G, Claustres M, Beroud C. Human Splicing Finder: an online bioinformatics tool to predict splicing signals. Nucleic Acids Res. 2009; 37:e67. [PubMed: 19339519]
63. Pitteloud N, Quinton R, Pearce S, Raivio T, Acierno J, Dwyer A, Plummer L, Hughes V, Seminara S, Cheng YZ, Li WP, Maccoll G, Eliseenkova AV, Olsen SK, Ibrahimi OA, Hayes FJ, Boepple P, Hall JE, Bouloux P, Mohammadi M, Crowley W. Digenic mutations account for variable phenotypes in idiopathic hypogonadotropic hypogonadism. J Clin Invest. 2007; 117:457–463. [PubMed: 17235395]
64. 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]
65. 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]
66. 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]
67. 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]
68. 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. 2011; 152:1616–1626. [PubMed: 21285314]
69. 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. 2010; 95:2276–2280. [PubMed: 20237166]

70. Donato J Jr, Cravo RM, Frazao R, Gautron L, Scott MM, Lachey J, Castro IA, Margatho LO, Lee S, Lee C, Richardson JA, Friedman J, Chua S Jr, Coppari R, Zigman JM, Elmquist JK, Elias CF. Leptin's effect on puberty in mice is relayed by the ventral premammillary nucleus and does not require signaling in Kiss1 neurons. J Clin Invest. 2011; 121:355–368. [PubMed: 21183787]
71. Roa J, Garcia-Galiano D, Varela L, Sanchez-Garrido MA, Pineda R, Castellano JM, Ruiz-Pino F, Romero M, Aguilar E, Lopez M, Gaytan F, Dieguez C, Pinilla L, Tena-Sempere M. The mammalian target of rapamycin as novel central regulator of puberty onset via modulation of hypothalamic Kiss1 system. Endocrinology. 2009; 150:5016–5026. [PubMed: 19734277]

Abbreviations

GnRH	gonadotropin-releasing hormone
LH	luteinizing hormone
FSH	follicle stimulating hormone

Table 1

Clinical characteristics and hormone profile of patients bearing biallelic mutations in *KISS1R*

				GnRH Challenge (Basal--Peak)		Treatment			
Patient	**Mutation (Biallelic) & Ref ID**	**History**	**Baseline**	**LH**	**FSH**	**T & E2**	**LH & FSH**	**GnRH**	**Outcome**
IV:6	L148S	Delayed puberty	LH< 1IU/L	↑ 40-50%	↑ 40-50%				Fertility
	#19, 47, 48	TV 3 cc	FSH 0.8 IU/L						
			T 3 ng/dL						
IV:2		Delayed puberty	LH 4.11IU/L	3--8.4 IU/L	2--4.6 IU/L		Y		Fertility
		TV 2 cc	FSH 2.4 IU/L						
			T 29 ng/dL						
IV: 4		1° amenorrhea					Y	Y	Fertility
IV: 5		“Short” penis	LH 0.5 1IU/L	0.5--3 IU/L	0.7--5 IU/L		Y		Fertility
		TV 1 cc	FSH 0.7 IU/L						
			T 14 ng/dL						
IV:15		TV 5 cc	LH 2.8 1IU/L						
		P2-3	FSH 3.7 IU/L						
			T 37 ng/dL						
IV:11		B3	LH 0.53 IU/L			Y			
		P4	FSH 2.55 IU/L						
			E2 28 ng/dL						
III.2	Del 155 bp	P3	T 26 ng/dL	1.5--3.6 mIU/mL	0.5--1.7 mIU/mL				
	# 18	Penis 7 cm							
III.3			T 19 ng/dL	1.5--1.4 mIU/mL	0.5--1.5 mIU/mL				
III.4			T 5 ng/dL	1.1--1.9 mIU/mL	4.1--4.1 mIU/mL				
III.6		Partial breast	E2 17 ng/dL	2.0--11.8 mIU/mL	3.4--6.4 mIU/mL				
III.7			T 5 ng/dL	2.6--3.4 mIU/mL	1.8--2.6 mIU/mL				
	R331X/X399R	↓ hair	LH 2.2 IU/L	2.4--12.7 mIU/mL	8.1--23.4 mIU/mL	Y		Y	Fertility
	# 19, 48	Penis 5 cm	FSH 3.2 IU/L						
		TV 1.2 mL	T 10 ng/dL						
	C223R/R297L	Microphallus	2 mo:	10 years:					

				GnRH Challenge (Basal--Peak)		Treatment			
Patient	**Mutation (Biallelic) & Ref ID**	**History**	**Baseline**	**LH**	**FSH**	**T & E2**	**LH & FSH**	**GnRH**	**Outcome**
	# 50	Cryptorchidism	LH<0.5mIU/mL FSH<0.5mIU/mL	<0.7--1.0 IU/L	<0.2--1.1 mIU/mL				
	1001-1002 insC # 44	Mild hypospadius Cryptorchidism	LH 1.1 IU/L FSH 2.2 IU/L T 3.4 nmol.L	peak 10.5 IU/L	peak 5.7 IU/L	Y	Y	Y	Fertility
Family I, I.1	L102P # 46	1° amenorrhea B4	LH 2.8 IU/L FSH 5.9 IU/L E2 24 pmol/L			Y		Y	Fertility
Sister		1° amenorrhea							
Brother		Delayed puberty							
Family II, II.1		Cryptorchidism	LH 0.5 IU/L FSH 1.62 IU/L T 0.1 ng/mL	0.5--2.5 IU/L Over 10 y: ↑LH/FSH	2.0--4.0 IU/L	Y		Y	
II.2		1° amenorrhea P5 B3	LH <0.5 IU/L FSH 1.1 IU/L E2 <50 ng/mL	0.5--0.5 IU/L	2.0--1.2 IU/L	Y			Bleeding
II.3		1 amenorrhea P3 B3	LH 1.3 IU/L FSH 4.0 IU/L E2 <50 ng/mL	1.5--8.5 IU/L	4.0--9.0 IU/L	Y			Bleeding
II.4		Cryptorchid TV 3 mL Penis 5 cm	LH <0.5 IU/L FSH <0.4 IU/L T 0.34 ng/mL			Y			
Older	IVS2 -4_-2 delGCA insACCGGCT	Microphallus Cryptorchidism	T 24 ng/dL	0.37--4.34 IU/L	2.3--6.39 IU/L				
Younger	# 45	Microphallus Cryptorchidism	LH <0.1 IU/L FSH 1.2 IU/L						
A1	F272S	TV 1.5 mL	T< 0.7 nmol/L	< 0.1--0.3 IU/L	0.45--2 IU/L				

				GnRH Challenge (Basal--Peak)		Treatment			
Patient	**Mutation (Biallelic) & Ref ID**	**History**	**Baseline**	**LH**	**FSH**	**T & E2**	**LH & FSH**	**GnRH**	**Outcome**
	# 49	Penis 5.5 cm							
A2		TV 0.5 mL							
		Penis 3.2 cm							
B1		TV 1 mL	T< 0.7 nmol/L	< 0.1--0.6 IU/L	0.4--3.6 IU/L				
		Penis 5 cm							
B2		TV 2 mL	T< 0.4 nmol/L	< 0.1--1.7 IU/L	0.6--2.7 IU/L				
		Penis 5.5 cm							
B3		TV 1 mL	T< 0.4 nmol/L	< 0.1--1.5 IU/L	0.8--4.0 IU/L				
B4			LH <0.07 IU/L						
			FSH 1.54 IU/L						
			E2 undetectable						