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Reproductive functions of kisspeptin and Gpr54 across the life cycle of mice and men

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Abstract

The reproductive phenotypes of nearly two dozen patients with mutations in *GPR54* have been reported, as have the phenotypes of four mouse lines mutant for *Gpr54* and two lines mutant for *Kiss1*. These phenotypes demonstrate that kisspeptin/Gpr54 function is required at all phases of the life cycle when the secretion of gonadotropin-releasing hormone (GnRH) is robust. Furthermore, there is phenotypic variability ranging from severe hypogonadism to partial sexual development. Collectively, these findings suggest that kisspeptin and Gpr54 serve as an essential conduit for relaying developmental information to the GnRH neuron.

Keywords

GPR54; *KISS1*; kisspeptin; reproduction; idiopathic hypogonadotropic hypogonadism

1. Introduction

The central role of GnRH in reproduction is well established, but the mechanisms that modulate GnRH neuronal secretion still need elucidation [15]. A critical window into understanding the regulation of GnRH secretion was opened with the discovery of the reproductive role of the kisspeptin/GPR54 signaling pathway. The link between kisspeptin/Gpr54 and GnRH secretion was discovered independently by several groups through two distinct genetic approaches: genetic studies of human patients with reproductive disorders [8,35], and reverse genetic studies in the mouse [13,35]. The ensuing intense study of the role of kisspeptin and Gpr54 in reproduction has rapidly advanced our understanding of the system's expression, physiology, pharmacology, and genetics. This review focuses on what has been learned about the reproductive functions of kisspeptin/Gpr54 across the life cycle from the study of human patients with mutations in *GPR54* and from mice carrying targeted deletions of *Kiss1* or *Gpr54*.

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2. The spectrum of phenotypes in patients with idiopathic hypogonadotropic hypogonadism

Mutations in *GPR54* were initially reported by two groups studying families affected by idiopathic hypogonadotropic hypogonadism (IHH) [8,35]. IHH is characterized by a failure of pubertal development, and affected patients show decreased levels of sex steroids associated with inappropriately low levels of gonadotropins. IHH can be caused by defects in GnRH secretion or action [38].

The study of patients with IHH has provided valuable insight into normal reproductive endocrine physiology. Though grouped under an umbrella diagnosis, patients with IHH have considerable variability in their presentation and hormonal profiles [4,28,38]. Some patients fail to exhibit any pubertal maturation; others enter puberty but fail to achieve complete sexual maturation. A small proportion have adult-onset IHH; these patients undergo normal sexual maturation as teenagers but develop IHH as adults [26]. The factors that lead to the dissipation of GnRH secretion in adult-onset IHH are as mysterious as the factors that lead to a failure of the activation of the HPG axis at the time of puberty in “classical” IHH.

Detailed neuroendocrine phenotyping of patients with IHH reveals further variability. Frequent blood sampling (every 10 minutes) is a powerful investigative tool used to detect the presence or absence of GnRH-induced luteinizing-hormone (LH) pulsations in patients with IHH. Some patients exhibit complete absence of LH pulsatility; others exhibit LH pulses but with low amplitude and/or frequency, demonstrating that their ability to secrete and respond to GnRH is still intact despite being markedly impaired; and yet others exhibit normal LH pulses at night but not during the day, similar to what is seen in early puberty and suggesting an even milder phenotype [38].

This phenotypic variation in the IHH patient population is an important backdrop for exploring the genetic factors that regulate the reproductive endocrine system. Increasing numbers of gene mutations are being discovered in patients with hypogonadotropic hypogonadism [7,9,12,23,24,29,31,43], including rare nucleotide variants in patients with the adult-onset form of IHH [5]. The spectrum of phenotypes varies between genes; for example, mutations in *KAL1* produce hypogonadotropic hypogonadism that is almost uniformly severe [34], whereas mutations in *FGFR1* produce much more variable phenotypes [29,34]. The precise molecular underpinnings for this variation remain unclear.

3. Phenotypes of human patients with mutations in *GPR54*

3.1 *GPR54* and reproductive function in the fetus and infant

The hypothalamic-pituitary-gonadal (HPG) axis is not only activated at puberty, but in fact has several ebbs and flows throughout prenatal and postnatal development [15]. GnRH secretion first appears in the second trimester of fetal development; sexual differentiation is largely complete at this time, but in males a functional HPG axis is required for testicular descent and penile growth. Defects in the HPG axis during the second and/or third trimesters may therefore result in a failure of testicular descent (cryptorchidism) and small penile size (microphallus) [14].

Many male patients with *GPR54* mutations reported in the literature were born with microphallus and/or cryptorchidism (Table 1). These include two individuals homozygous for the mutation L102P [42], one individual who is a compound heterozygote with the mutations C233R and R297L [36], and an individual homozygous for the mutation 1001_1002insC [21]. The patient with the 1001_1002insC mutation was also noted to have a mild hypospadias. Both the L102P and the C233R mutations abrogate Gpr54 signaling in vitro [36,42], while the

R297L mutation results in a milder reduction in signaling activity [36]. While the effects of the 1001_1002insC mutation on Gpr54 signaling have not been reported, the mutation is likely to result in a significant impairment in Gpr54 signaling since the frameshift caused by the insertion results in a complete alteration of the cytoplasmic tail of the receptor [21]. The cryptorchidism and micropallus of these patients, and the hypospadias of the one patient, demonstrate that the kisspeptin/Gpr54 system is required for HPG axis activity in the developing male fetus.

GnRH secretion decreases at birth but resumes within the first few days of life and persists for 6 months to 2 years [15]. This results in sex-steroid levels comparable to those of adults, though for unknown reasons secondary sex characteristics do not emerge. The function of this “minipuberty” of infancy remains unclear, although some data suggests that in males it potentiates future spermatogenic function and fertility [37].

While the HPG axis is active during both puberty and the minipuberty of infancy, the mechanisms of activation appear to be distinct. Patients with mutations in *NROB1* (also called *DAX1*) have adrenal hypoplasia congenita, a condition characterized by adrenal insufficiency and hypogonadotropic hypogonadism [44]. (Gain-of-function mutations in *NROB1* cause defects in testicular development, resulting in sex reversal.) Patients exhibit IHH at the time of puberty but have normal HPG activity as infants [1,25,40]. Similarly, boys with Prader-Willi syndrome, which is caused by imprinting defects or chromosomal abnormalities at 15q11-12, also have a normal minipuberty of infancy but later develop IHH [10]. The preservation of the minipuberty of infancy in these patients who later develop IHH suggests that distinct mechanisms regulate the HPG axis during these two developmental stages. Alternatively, the hypogonadism of these patients may be a slowly progressive phenomenon.

An important insight into the neonatal role of Gpr54 comes from gonadotropin levels measured in the individual with the C233R/R297L mutations in *GPR54* [36]. This patient’s gonadotropin levels were found to be undetectable at 2 months, a time when they should have been comparable to adult levels. Since patients with mutations in *GPR54* have abnormalities in both puberty and the minipuberty of infancy, kisspeptin/GPR54 signaling appears to be a common pathway used during both infancy and puberty to stimulate GnRH secretion.

3.2 *GPR54* and the pubertal transition

Studies of patients with *GPR54* mutations have firmly established the importance of the kisspeptin/Gpr54 system for puberty, with several groups reporting IHH in patients with homozygous or compound heterozygous loss-of-function mutations in *GPR54* [8,21,35,36, 42]. Though these mutations are generally severe, the patients exhibit variable degrees of hypogonadism, with some exhibiting complete absence of pubertal development and others experiencing partial, incomplete puberty (Table 1). To date, more girls than boys have been described to have partial pubertal development (i.e., breast development), even though many carry the same mutations.

Two frequent sampling studies of patients with *GPR54* mutations have been reported [35,42]. One was performed in the patient homozygous for the mutation L102P, which abolishes receptor signaling in vitro as noted in Section 3.1, the other in a patient who carries compound heterozygous mutations in *GPR54* that result in nonsense and nonstop transcripts (R331X and X399R). Reverse transcription-polymerase chain reaction (RT-PCR) studies demonstrated very low levels of both transcripts in immortalized white blood cells, and the mutant Gpr54 isoforms produced by these transcripts were shown to be incapable of stimulating second-messenger signaling in vitro. In both these patients, frequent blood sampling revealed the presence of LH pulsations, though of very low amplitude, demonstrating the persistence of GnRH pulse generation despite the patients’ severe *GPR54* mutations.

GnRH-stimulation testing is commonly used to assess pituitary responsiveness and has been performed in multiple patients with *GPR54* mutations [21,27,35,36,42]. Patients' responses to exogenous GnRH were variable, ranging from subnormal to normal to robust. Since the pituitary requires prior exposure to GnRH to exhibit a normal response, these findings provide further evidence that some patients with *GPR54* mutations are still capable of endogenous GnRH secretion.

In one patient, multiple GnRH-stimulation tests were performed over several years [42]. While the patient's initial responses showed a greater rise in follicle-stimulating hormone (FSH) than LH levels, over time the rise in LH levels became more predominant. This shift from an FSH-predominant to an LH-predominant response occurs across normal puberty, and was preserved in this patient despite her mutations in *GPR54*.

Since loss-of-function mutations in *GPR54* result in failure to undergo a normal puberty, gain-of-function mutations might result in the opposite phenotype: precocious puberty. A novel heterozygous change in *GPR54*, R386P, was identified in an adopted girl with idiopathic GnRH-dependent precocious puberty [41]. In vitro studies demonstrated that the R386P mutation leads to prolonged activation of intracellular signaling pathways in response to kisspeptin. For both wild-type and R386P *Gpr54*, inositol phosphate levels peaked at 2 hours, but the subsequent rate of decline in IP levels was slower with R386P. These findings indicated a significant reduction in the rate of receptor desensitization for the mutant receptor. The association of *GPR54* loss-of-function mutations with IHH and a gain-of-function mutation with precocious puberty support the concept of kisspeptin and *Gpr54* as critical modulators of the HPG axis at puberty in man.

3.3 Fertility in patients with *GPR54* mutations

Patients with mutations in *GPR54* can achieve fertility through hormone replacement. The patient with the R331X/X399R mutations and the patient homozygous for 1001_1002insC underwent long-term therapy with exogenous pulsatile GnRH, achieved spermatogenesis, and fathered children [21,27]. Three affected males with homozygous L148S mutations underwent treatment with exogenous gonadotropins, resulting in testicular growth, spermatogenesis, and subsequent fertility [27]. Therefore, mutations in *GPR54* do not preclude the development of mature sperm.

A female patient homozygous for the L102P mutation achieved pregnancy with treatment with exogenous gonadotropins [42]. Another female patient, homozygous for the L148S mutation, had 1) an intact response to exogenous GnRH and gonadotropins, 2) multiple conceptions, 3) two uncomplicated pregnancies and deliveries of healthy children, 4) spontaneous initiation of uterine contractions, and 5) lactation for several months post-partum [27]. Though *KISS1* and *GPR54* are expressed at high levels in the placenta [2,18], these pregnancies demonstrate that maternal *GPR54* function is not required for normal placental function, just as the normal births of patients homozygous for mutations in *GPR54* show that fetal *GPR54* function is not strictly required for normal placentation.

3.4 Pituitary and gonadal phenotypes

Expression of *GPR54* has been noted in the pituitary gland and gonads, but its exact role in these tissues is unclear [16,33,39]. The endocrine phenotyping of patients with *GPR54* mutations has hinted at actions of kisspeptin/*Gpr54* in the pituitary and the gonad.

The patient with the R331X/X399R *GPR54* mutations underwent a dose-response study using exogenous GnRH while he was receiving testosterone replacement therapy [35]. The amplitudes of his LH responses to four different doses of intravenous GnRH were compared

to those of six other IHH men who do not carry mutations in *GPR54*; the dose-response curve for the index patient was shifted significantly leftward. It is unclear why this patient was more sensitive to exogenous GnRH, particularly since in vitro data suggests that, if anything, kisspeptin acts at the pituitary to potentiate gonadotropin release [16,39].

The patient with the C223R/R297L *GPR54* mutations underwent human chorionic gonadotropin (hCG) stimulation testing at ages 15 months and 10 years [36]. At both times his testosterone response was subnormal. The kisspeptin/GPR54 system may therefore have a direct effect on testicular function, though his impaired response may be a secondary result of his hypogonadotropic hypogonadism.

3.5 Human subjects with heterozygous mutations in *GPR54*

Little has been reported on subjects who carry heterozygous mutations in *GPR54*. The mother and brother of the patient with the C233R/R297L mutations were both heterozygous for the R297L mutation [36]. Both had no apparent reproductive endocrine defects. As noted in section 3.1, the R297L mutation results in a mild attenuation of Gpr54 activity. The phenotypes of individuals heterozygous for severe mutations in *GPR54* have not been described in detail; though pedigrees show that these heterozygotes can bear children, subtle defects in the menstrual cycling or fertility of these individuals may have gone unnoticed.

4. Phenotypes of *Gpr54* and *Kiss1* mutant mice

At least four *Gpr54* mutant mouse models have been generated [13,20,22,35], and the phenotypes of two *Kiss1* mutant mouse models have been reported [6,21]. In general, the phenotypes of both *Gpr54* and *Kiss1* mice parallel the phenotypes of patients with mutations in *GPR54*, with abnormal sexual maturation at a variety of developmental time points.

4.1. GnRH secretion in mice

The pattern of GnRH secretion in rodents is sexually dimorphic [17]. In male mice, the fetal and neonatal patterns of secretion resemble those of humans, and dysfunction of the HPG axis results in failure of testicular descent and microphallus. There is a surge of HPG axis activity on the first day of life of male rodents, and this surge has been implicated in establishing sexually dimorphic differences in neuroanatomy and behavior. The HPG axis remains relatively quiescent during the juvenile period; however, sex-steroid feedback appears to play a much greater role in this attenuation of GnRH secretion in the rodent than in humans [32]. Then, at sexual maturation, the HPG axis becomes active once again in males and apparently for the first time in females. Sexual maturation is evidenced in males by separation of the prepuce from the penis, lengthening of the anogenital distance, and the initiation of spermatogenesis, and in females by the opening of the vaginal orifice and the initiation of estrus cyclicity, folliculogenesis, and ovulation.

4.2 Fetal and neonatal effects

Similar to human patients, *Gpr54*^{-/-} and *Kiss1*^{-/-} mice exhibit no defects in early fetal development: no differences in reproductive organs were seen at embryonic day 16.5 (E16.5) [13,35], and mutant mice do not exhibit sexual ambiguity at birth, with litters having normal ratios of male to female pups [22]. The anogenital distance of newborn male *Gpr54*^{-/-} pups is not different from that of their wild-type littermates [20]; this finding shows that Gpr54 signaling is not required for the emergence of this sexually dimorphic trait, most likely because it is established prior to GnRH-dependent production of testosterone. By postnatal day 21 (P21), however, the anogenital distance (AGD) of *Gpr54*^{-/-} males is shorter than that of wild-type animals, though this difference reached statistical significance in only one of two reports [20,22]. Furthermore, P21 male *Gpr54*^{-/-} animals have significantly smaller penises [20]. These

findings suggest that the kisspeptin/Gpr54 pathway functions during neonatal development in male mice as it does in humans.

4.3 Juvenile and adult effects

During sexual maturation, the phenotypes of *Gpr54*^{-/-} and *Kiss1*^{-/-} mice become even more apparent, but different degrees of hypogonadism have been reported in different mouse models.

Male mutant mice have greatly reduced rates of preputial separation, and their anogenital distance fails to lengthen to the same extent as that of WT mice [22]. Testicular size is reduced [6,13,20,22,35], and gonadotropin and testosterone levels are low [6,22,35]. Mice are infertile, due at least in part to defects in sexual behavior; the sexual behaviors can be restored by treatment with exogenous testosterone [20]. Varying degrees of spermatogenesis have been reported [6,22,35]. Mice with mutations in *Gpr54* have impairment of spermatogenesis that ranges from complete to only moderate, even within the same line [6,22,35]. In one *Kiss1* mutant line, spermatogenesis was markedly abrogated [6], whereas in another line spermatogenesis was only mildly to moderately impaired [22].

Variable hypogonadism has also been observed in female mice. One group observed a consistently severe phenotype in both *Gpr54*^{-/-} and *Kiss1*^{-/-} mice, with vaginal smears consistent with sexual immaturity and folliculogenesis arrested at the antral stage [6,35]. Another group observed that only a subset of both *Gpr54*^{-/-} and *Kiss1*^{-/-} females remain sexually immature, with vaginal smears suggesting anestrus/diestrus, uterine weights greatly reduced, and folliculogenesis arrested at the antral stage [22]. In contrast, other females achieve partial sexual maturation, with vaginal smears showing persistent estrus, normal uterine weights, and ovarian histology showing tertiary follicles or multiple large cysts containing no obvious oocytes. Despite these differences in phenotype, all female mice fail to ovulate, as demonstrated by lack of corpora lutea, and are infertile. Female mice also lack sexual behaviors, which can be rescued by replacement of estradiol and progesterone [20].

These differences in severity of phenotype may be due to differences in strain or in construction of mutant alleles. Though the exact cause is unclear, this variability illustrates that disruption of kisspeptin/Gpr54 signaling can result in a range of phenotypes, even among animals of the same strain. Thus, in both mice and men, partial sexual maturation can still occur despite mutations that severely compromise kisspeptin/Gpr54 signaling.

5. Conclusions

Mutations disrupting the kisspeptin/Gpr54 system produce a range of phenotypes in both mice and men. This phenotypic variability could be due to differences in severity of mutations, though this variability can be seen even between patients with the same mutations in *GPR54*. Mutations in more than one gene have recently been found to explain the variability in reproductive phenotypes within a kindred [9,30], though no examples involving *GPR54* or *KISS1* have been described to date. Environmental influences and stochastic variation may further contribute to phenotypic variability. This variability suggests that the range of human phenotypes caused by mutations in *GPR54* and/or *KISS1* may extend beyond IHH and include milder reproductive conditions. Careful phenotyping may also reveal subtle reproductive phenotypes in patients with heterozygous mutations in *GPR54* or *KISS1*.

The mutant phenotypes of mice and men indicate that the kisspeptin/Gpr54 system is required at all times when the GnRH neuron is active: fetal development, neonatal life, puberty, and adulthood. Furthermore, a number of physiological inputs, such as sex steroid feedback and effects of energy balance, have been shown to modulate GnRH activity at least in part through their effects on kisspeptin expression [19]. The kisspeptin/Gpr54 system therefore appears to

be the primary interface between the GnRH neuron and developmental and physiological inputs. Future work will undoubtedly reveal how the kisspeptin neuron integrates these various signals.

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Table 1

Reproductive, laboratory, and molecular characteristics of patients with mutations in *GPR54*. GnRH, gonadotropin-releasing hormone; LH, luteinizing hormone; FSH, follicle-stimulating hormone; hCG, human chorionic gonadotropin.

Reference	Mutation	In vitro activity	Sex	Phenotype and Laboratory Evaluation
[8]	c.738-14-c.880 del.	not assessed	M	<ul style="list-style-type: none"> At 20 y, testes 4 ml, penis 7 cm, pubic hair Tanner 3. After GnRH stimulation, LH 1.4 U/L, FSH 1.5 U/L. At 21 y, similar clinical features. After GnRH stimulation, LH 3.6 U/L, FSH 1.7 U/L. At 19 y, similar clinical features. After GnRH stimulation, LH 1.9 U/L, FSH 4.1 U/L. At 14 y, similar clinical features. After GnRH stimulation, LH 3.4 U/L, FSH 2.6 U/L.
			F	<ul style="list-style-type: none"> At 16 y, partial breast development, single episode of uterine bleeding. At 18 y, after GnRH stimulation, LH 11.8 U/L, FSH 6.4 U/L.
[3,27]	L148S	greatly reduced	M	<ul style="list-style-type: none"> Delayed puberty testes 3 ml. After GnRH stimulation, 40-50% increase in gonadotropins. Fertility with gonadotropin therapy. Delayed puberty, testes 2 ml. After GnRH stimulation, LH 8.4 U/L, FSH 4.6 U/L. Fertility with gonadotropin therapy. Testes 1 ml, short penis. After GnRH stimulation, LH 3 U/L, FSH 5 U/L. Fertility with gonadotropin therapy. Delayed puberty, testes 5 ml, pubic hair Tanner 2-3.
			F	<ul style="list-style-type: none"> Primary amenorrhea. Fertility with pulsatile GnRH. Primary amenorrhea, breasts Tanner 3, pubic hair Tanner 4.
[27,35]	R331X/X399R	greatly reduced	M	<ul style="list-style-type: none"> At 17 y, testes 1 ml, penis 5 x 1.75 cm. After GnRH stimulation, LH 12 U/L, FSH 23 U/L. At 28 y, low amplitude LH pulses. Fertility with pulsatile GnRH therapy.
[42]	L102P	greatly reduced	M	<ul style="list-style-type: none"> At birth, bilateral cryptorchidism. At 21 y, testes 3-4-ml, pubic hair Tanner 3, penis 7.2 cm. After GnRH stimulation, LH 5.5 U/L, FSH 3 U/L. Bilateral cryptorchidism at birth. At 32 y, 3 ml testes, 5 cm penile length, azospermia, pubic hair Tanner 5, LH<0.5 U/L, FSH<0.5 U/L.
			F	<ul style="list-style-type: none"> At 27 y, primary amenorrhea, breasts Tanner 4. After GnRH stimulation, LH 32 U/L, FSH 17 U/L. Low-amplitude LH pulses. Fertility with gonadotropin therapy.

Reference	Mutation	In vitro activity	Sex	Phenotype and Laboratory Evaluation
				<ul style="list-style-type: none">At 17 y, primary amenorrhea, breasts Tanner 3, pubic hair Tanner 5.At 16 y, primary amenorrhea, breasts Tanner 3, pubic hair Tanner 3.
[36]	C233R/R297L	greatly/slightly reduced	M	<ul style="list-style-type: none">At birth, micropenis & cryptorchidism. At 2 mo, undetectable gonadotropins. At 15 mo, with hCG stimulation, testosterone <15 ng/dL → 45 ng/dL. At 10 y, after GnRH stimulation, LH 1.0 U/L, FSH 1.8 U/L; with hCG stimulation, testosterone 21 ng/dL → 140 ng/dL.
[21]	1001_1002in sC	not assessed	M	<ul style="list-style-type: none">At birth bilateral cryptorchidism, mild hypospadias. At 20 y, after GnRH stimulation, LH 10.5 U/L, FSH 5.7 U/L. At 32 y, right testis 3 ml, left 5.3 ml by ultrasound. Fertility with pulsatile GnRH.
[41]	A386P	less desensitization	F	<ul style="list-style-type: none">At age 8, enlarged ovarian and uterine mass for chronological age, pubic hair Tanner 2, breasts Tanner 4. After GnRH stimulation, LH 6.4 U/L, FSH 5.9 U/L.