Kiss1−/− Mice Exhibit More Variable Hypogonadism than Gpr54−/− Mice

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The G protein-coupled receptor Gpr54 and its ligand metastin (derived from the Kiss1 gene product kisspeptin) are key gatekeepers of sexual maturation. Gpr54 knockout mice demonstrate hypogonadotropic hypogonadism, but until recently, the phenotype of Kiss1 knockout mice was unknown. This report describes the reproductive phenotypes of mice carrying targeted deletions of Kiss1 or Gpr54 on the same genetic background. Both Kiss1 and Gpr54 knockout mice are viable but infertile and have abnormal sexual maturation; the majority of males lack preputial separation, and females have delayed vaginal opening and absence of estrous cycling. Kiss1 and Gpr54 knockout males have significantly smaller testes compared with controls. Gpr54 knockout females have smaller ovaries and uteri than wild-type females. However, Kiss1 knockout females demonstrate two distinct phenotypes: half have markedly reduced gonadal weights similar to those of Gpr54 knockout mice, whereas half exhibit persistent vaginal cornification and have gonadal weights comparable with those of wild-type females. FSH levels in both Kiss1 and Gpr54 knockout males and females are significantly lower than in controls. When injected with mouse metastin 43–52, a Gpr54 agonist, Gpr54 knockout mice fail to increase gonadotropins, whereas Kiss1 knockout mice respond with increased gonadotropin levels. In summary, both Kiss1 and Gpr54 knockout mice have abnormal sexual maturation consistent with hypogonadotropic hypogonadism, although Kiss1 knockout mice appear to be less severely affected than their receptor counterparts. Kiss1 knockout females demonstrate a bimodal phenotypic variability, with some animals having higher gonadal weight, larger vaginal opening, and persistent vaginal cornification. (Endocrinology 148: 4927–4936, 2007)
Fig. 1. Targeted disruption of Kiss1 and Gpr54 genes. A and D, Schematic diagrams of the targeting vectors, the endogenous loci, and the disrupted loci of Kiss1 (A) and Gpr54 (D). B and E, Screening of ES cells by PCR using one primer located outside of the 5′ or the 3′ end of the targeting vector and a second primer in the Neo gene (arrows) generates 4.9- and 5.7-kb products in the targeted Kiss1 ES cells (B) and 5.2- and 5.4-kb products in the targeted Gpr54 ES cells (E). Southern blot analysis of BssS1- or Xba1-digested DNA using a probe outside of the targeting vector detected 6.9- and 13-kb fragments (B) or 7.2- and 16-kb fragments (E) in the targeted locus and in the endogenous locus, respectively. C and F, PCR amplified a fragment of 453 bp in WT (+/-) mice, fragments of 453 and 1205 bp in heterozygous (+/-) mice, and a fragment of 1205 bp in Kiss1 mutant (-/-) mice (C) and a fragment of 1566 bp in WT (+/-) mice, fragments of 1566 and 2051 bp in heterozygous (+/-) mice, and a fragment of 2051 bp in Gpr54 mutant (-/-) mice (F). RT-PCR using one primer in exon 1 and a second primer in the last end of the targeting gene amplified a 340-bp fragment or 1229-bp fragment in both WT (+/-) and heterozygous (+/-) mice but not in Kiss1 (C) or Gpr54 (F) mutant (-/-) mice, respectively. β-Actin was used as a control.

Resulting chimeric males were crossed with 129/S1/SvImJ females for germline transmission of the mutated alleles. The resulting male and female heterozygotes were bred to generate wild-type (WT), heterozygous, and knockout animals for each targeted deletion. Genotyping was performed with genomic DNA isolated from a piece of tail using the DNeasy Tissue Kit (QIAGEN, Valencia, CA) and PCR with primers listed in supplemental Fig. 1.

Animal housing

All mice were kept in cages of one to five animals and housed under a 12-h light cycle and controlled temperature in the experimental animal facility of the Massachusetts General Hospital Center for Comparative Medicine. Food and water were available ad libitum. All procedures were approved by the Subcommittee on Research Animal Care of Massachusetts General Hospital.

Rapid amplification of cDNA ends (5′-RACE) analysis

The DNeasy kit (QIAGEN) was used to isolate cDNA from hypothalami of Kiss1 knockout and WT mice. The SMART 5′-RACE kit (Clontech, Mountain View, CA) was used to amplify the cDNA of Kiss1 transcription products using a primer in the last exon of Kiss1 (primer sequence in supplemental data). The products of RACE were cloned using the TOPO TA cloning kit (Invitrogen, Carlsbad, CA) for sequence analysis.

Immunohistochemistry for Kiss1 knockout mice

Kiss1 knockout and WT mice were anesthetized with ketamine and xylazine and perfused with 2.5% acrolein plus 4% paraformaldehyde in phosphate buffer. After perfusion, the brain was removed from the skull and sunk in 30% aqueous sucrose solution. Sections were cut at 28 μm with a freezing microtome into cryoprotectant antifreeze solution (8) into 12 serial series and stored at −20°C until they were processed. The kisspeptin antibody was the gift of Dr. Alain Caraty and Dr. Isabella Franceschini-Laurent, Unité de Physiologie de la Reproduction et des Comportements, University of Tours, France (9), and was used at a dilution of 1:300,000. Staining was performed on a one-in-six series of sections using conventional avidin-biotin complex techniques as described previously (10) and visualized using a nickel sulfate-diaminobenzidine chromogen solution. After reaction with the chromogen solution for 15 min, the sections were rinsed in acetate solution followed by PBS, placed into normal saline, mounted onto glass slides, dried overnight, dehydrated, and coverslipped. Sections were examined with a Nikon E800 microscope, photographed using a Retiga-EX cooled CCD digital camera, and captured onto a Macintosh G4 computer using Vision-Mac (BioVision Technologies, Exton, PA).
Phenotyping
Details of births, including litter size, abnormalities, and neonatal deaths, were recorded. The mice were weaned and genotyped at 3 wk of age and then weighed and inspected two to three times per week. Sexual maturation was assessed by inspecting the presence or absence of vaginal opening (females) or preputial separation (males) and by measuring the anogenital distance (males) (11). Vaginal smears were obtained using a calcium alginate swab wetted with PBS, applied to a slide, fixed with 95% ethanol, and then stained using the Hema3 system (Fisher Scientific, Pittsburgh, PA). For fertility assessment, a knockout animal of each gender was placed in a cage with a WT mouse of the opposite gender and of proven fertility, and females were observed for evidence of pregnancy.

The 9- to 16-wk-old animals were weighed, inspected, and then killed by asphyxiation with carbon dioxide. Some mice received a single injection of 50 nmol C-terminally amidated mouse kisspeptin 110–119 (corresponding to metatin 43–52 and also known as kisspeptin-10) in PBS sc 30 min before being killed. Some mice were injected with the vehicle only. Blood was obtained by cardiac puncture, and organs were removed, inspected, and weighed. All phenotyping experiments were done without the knowledge of the genotype.

Tissue preparation and analysis
Serum was stored at −80 C. For sperm analysis, a single epididymis was dissected, weighed, and diced in 0.5 ml PBS with 4 mg/ml BSA and then incubated at room temperature. Ten microliters were applied to a hemocytometer for sperm to be counted. A separate sample was examined on a Petri dish for sperm motility.

Tissues for histology were fixed in Bouin’s solution (testes and epididymides) or 4% paraformaldehyde in PBS (other tissues) and then stored in 70% ethanol until processing. Tissues were embedded in paraffin, sectioned, and stained with hematoxylin and eosin by the Rodent Histopathology Core facility at Harvard Medical School. Samples for RNA analysis were stored in RNAalater (Ambion, Austin, TX) or snap frozen and stored at −80 C.

Hormone assays
All hormone assays were performed by the Ligand Assay Core of the Specialized Cooperative Center for Research in Reproduction at the University of Virginia. The reportable range of the LH assay was 0.04–37.4 ng/ml, and the intra and interassay coefficients of variation were less than 7 and 9%. The reportable range of the FSH assay was 3.4–35.9 ng/ml, and the intra and interassay coefficients of variation were less than 8 and 10%. The reportable range of the testosterone assay was 7.0–801.0 ng/dl, and the intra and interassay coefficients of variation were less than 7 and 9%.

Statistical analysis
All data are reported as mean ± sd unless otherwise stated. Differences between groups of heterozygous and WT animals were analyzed with ANOVA. No significant differences were observed, and no post hoc analyses were performed. In contrast, differences between groups of homozygous mutant and WT animals were analyzed with the unpaired Mann-Whitney U test because of the nonnormal distribution of data. The χ2 test was used in the analysis of vaginal opening and in the analysis of sex and genotype ratios. To avoid false positives, Bonferroni correction was applied when multiple testing between groups occurred. The P values reported are the corrected P values, and P values < 0.05 are considered significant. Comparison is with the WT group of the same sex unless otherwise stated. All mice were treated as independent of littermate relationships.

Results
Targeted disruption of Kiss1 and Gpr54
Constructs targeting the first exon of Kiss1 and the second exon of Gpr54 for replacement with the Neo cassette were created and used for homologous recombination in ES cells (Fig. 1). PCR and Southern blotting confirmed that recombinant cells carried the targeted deletion with no unintended insertions of the targeting construct. Recombinant cells were injected into blastocysts and the resulting chimeras were used to generate mice heterozygous for the targeted deletion. Heterozygote mice of both lines have litters at the same frequency and of the same size as WT 129 mice.

RT-PCR using primers directed against exon 2 of Kiss1 did amplify a transcript in Kiss1 knockout mice. To characterize this transcript, RACE and sequencing of Kiss1 transcripts were performed on WT and Kiss1 knockout mice. Transcripts isolated from hypothalami of WT mice revealed a new exon that lies 5′ to the previously known exon 1, with two splice acceptor sites within the previous exon 1 resulting in two transcripts with slightly differing 5′ untranslated regions but identical coding regions (supplemental data). Twenty RACE products isolated from the hypothalami of Kiss1 knockout mice were the same size. Five were sequenced and found to be identical. This transcript consists of the newly identified 5′-most exon spliced to exon 2. This transcript contains no in-frame translation start site and is therefore predicted to be incapable of producing functional kisspeptin.

To further confirm loss of kisspeptin expression in the Kiss1 knockout animals, immunohistochemistry was performed on hypothalamic sections with an antibody against kisspeptin. Kisspeptin was clearly expressed in WT hypothalamic slices in the arcuate and anteroventral periventricular nuclei, consistent with previous descriptions (12–14), but no staining was seen in hypothalamic slices of Kiss1 knockout mice (Fig. 2).

Fig. 2. Immunohistochemistry of kisspeptin in the hypothalamus. A–D, Immunocytochemical localization of kisspeptin in WT (A and B) and knockout (C and D) mice. A micrograph of the anteroventral periventricular nuclei of a female WT mouse shows the cells for kisspeptin as well as abundant axons within the periventricular region (A) (males have a few neurons and fewer axons, data not shown). In the same region of the Kiss1 P knockout mouse, no kisspeptin is detected (B). In the arcuate nucleus, the abundant axons for kisspeptin are easily seen in both female (C) and male WT mouse. Kiss1 knockout mice show absence of kisspeptin immunoreactivity in the arcuate (D). Bar, 100 μm.
Characterization of Kiss1 and Gpr54 null mice

Kiss1 and Gpr54 null mice are viable. Knockout adult animals display normal feeding and motor activity; mating behavior was not observed in any cages during routine animal husbandry. Sexual assignment was unambiguous for homozygous mutants of both targeted genes, and males and females were born in expected ratios (supplemental data). Kiss1 heterozygous crosses produced offspring in expected Mendelian ratios, but Gpr54 heterozygous crosses produced fewer Gpr54 homozygous offspring than expected, probably due to a slight skewing of gamete ratios (supplemental data).

The body weight of Gpr54 knockout males, but not Kiss1 males, was slightly but significantly less than that of WT males at 9–12 wk (Kiss1−/− 22.1 ± 2.3 g, nonsignificant; Gpr54−/− 20.4 ± 1.8 g, P < 0.001; WT 23.4 ± 2.3 g; Table 1). In contrast, the body weights of Kiss1−/− and Gpr54 knockout females were not significantly different from controls (Kiss1−/− 20.2 ± 1.8 g, Gpr54−/− 21.6 ± 3.5 g, WT 19.4 ± 1.8 g; Table 1).

Underdevelopment of the genitalia was the only gross abnormality noted on external examination of adult knockout mice. Males of both knockout lines largely lacked preputial separation (present in two of nine of 7- to 31-wk-old Kiss1−/− males and zero of eleven of 8- to 28-wk-old Gpr54−/− males) and had shorter anogenital distances than WT males (Kiss1−/− 13 ± 1 mm, P < 0.001; Gpr54−/− 13 ± 1 mm, P < 0.001; WT 17 ± 2 mm; Fig. 3B and Table 1). Heterozygous males were indistinguishable from WT males, and preputial separation always occurred by 5 wk of age. Both showed preputial separation as expected. Anogenital distance, a marker of androgen exposure (11), in both lines of knockout male mice diverged from that of WT animals early in the course of sexual maturation (d 23–25 of life) (Fig. 3B).

In females, vaginal opening was significantly delayed in both lines (Kiss1−/− P < 0.005; Gpr54−/− P < 0.001, Fig. 3D). The delayed vaginal opening of the knockout female mice was not attributable to differences in body weight, because both WT and knockout females followed the same weight trajectory (Fig. 3C). Some animals not included in the study shown in Fig. 3D had no vaginal opening even by 9–13 wk of age, but most animals ultimately achieved this milestone (Fig. 3D). The vaginal orifices of the adult knockout animals were often small and round compared with those of WT or heterozygous animals. Dye injection confirmed normal anatomic connectivity to the uterus. Vaginal smears of 9- to 11-wk-old Kiss1 and Gpr54 knockout female mice showed absence of estrous cycling. Nearly all Gpr54 null mice and almost half of Kiss1 knockout mice had little cellularity on vaginal smears. However, more than half of the Kiss1 knockout mice and one Gpr54 knockout mouse exhibited persistent vaginal cornification; these animals had vaginal openings that resembled those of WT females.

At necropsy, internal organs of adult mice (heart, lungs, liver, kidney, spleen, stomach, pancreas, and gut) had similar weights and appearance irrespective of genotype. Knockout males had small testes in comparison with WT mice (Kiss1−/− testis 39.0 ± 14.8 mg, P < 0.001; Gpr54−/− testis 24.2 ± 10.8 mg, P < 0.001; WT testis 141.0 ± 17.8 mg; Fig. 4A and Table 1). Furthermore, the reduction was significantly more severe in Gpr54 males than in Kiss1 males (P < 0.05). Sex-hormone-dependent organs such as seminal vesicles and preputial glands were small and sometimes difficult to detect. Heterozygous animals were not significantly different from WT animals. The female reproductive hemi-block (half of the uterus, one oviduct, and one ovary) was significantly smaller in Gpr54 knockout females compared with WT females but not in Kiss1 knockout females when compared with WT females as a group (Kiss1−/− 39.1 ± 27.0 mg, nonsignificant; Gpr54−/− 20.1 ± 23.4 mg, P < 0.001; WT 48.5 ±

TABLE 1. Data for all mice grouped by genotype

<table>
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<tr>
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<th>Gpr54−/−</th>
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<th>WT</th>
<th>Kiss1−/−</th>
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<tr>
<td></td>
<td>Mean ± SEM n</td>
<td>Mean ± SEM n</td>
<td>Mean ± SEM n</td>
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<td>47.1 ± 3.7 16</td>
<td>48.5 ± 3.6 26</td>
<td>54.1 ± 6.3 18</td>
<td>39.1 ± 7.2 14</td>
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<td>VO age (d)</td>
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<td>28.7 ± 0.5 15</td>
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<td>4.3 ± 1.0 6</td>
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<td>3.8 ± 0.9 8</td>
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<td>23.4 ± 0.6 17</td>
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<td>7.0 ± 0.4 7</td>
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Gonadal weight is the average of testes weights for males and average of weights for genital hemiblock (ovary, oviduct, and half of uterus) for females. AGD, Anogenital distance; Stim., animals stimulated 30 min before being killed with 50 nmol metasin C-terminal decapetide sc; T, testosterone; VO, vaginal opening.

a Statistically significant difference (corrected P < 0.05) when compared with the WT group.
b Statistically significant difference (corrected P < 0.05) between Gpr54 and Kiss1 knockout mice.
The gonadal weight of Kiss1 knockout females followed a bimodal distribution. One group had a mean gonadal weight of 11.6 ± 2.3 mg, similar to Gpr54 knockout females and significantly different from WT females (P < 0.001). The other group had a mean gonadal weight of 59.8 ± 14.8 mg, similar to that of WT females. Kiss1 and Gpr54 heterozygotes were not significantly different from WT animals.

Gonadal histology

The seminiferous tubules and interstitial cells of null males of both lines had normal histological architecture. However, although early stages of spermatogenesis appeared normal, mature spermatozoa were either absent or present in reduced numbers in mutant mice of both lines (Fig. 5, A–F). Leydig cell density was not overtly different between knockout and WT lines. Because the testes of knockout animals were significantly smaller, this suggests that total Leydig cell number was reduced in the null animals. Epididymides of knockout males contained less sperm and more nonsperm cells than epididymides of WT or heterozygous males (Fig. 5, A–F).

Ovaries from knockout females of both lines displayed follicles up to the antral stage of development, but no preovulatory follicles or corpora lutea were seen. In addition, many atretic follicles were observed in mutant ovaries (Fig. 5, G–L). Kiss1 knockout females with persistent vaginal cornification had larger ovaries that contained multiple large cysts, but no sign of ovulation was seen (Fig. 5, G–L).

Serum gonadotropin levels with and without kisspeptin stimulation

Baseline levels of LH and FSH were obtained at dissection of adult mice. In both sexes and both lines, FSH was reduced in knockout animals compared with WT mice (males: Kiss1/−/− 1.9 ± 1.2 ng/ml, P < 0.001; Gpr54/−/− 1.5 ± 0.5 ng/ml, P < 0.001; WT 20.5 ± 8.8 ng/ml; females: Kiss1/−/− 3.8 ± 2.5 ng/ml, nonsignificant; Gpr54/−/− 3.3 ± 1.8 ng/ml, P < 0.05; WT 6.0 ± 2.3 ng/ml; Fig. 6, C and D, and Table 1). However, only Gpr54 knockout males had significantly reduced LH levels (Gpr54/−/− 0.05 ± 0.02 ng/ml, P < 0.001; WT 0.20 ± 0.16 ng/ml). Kiss1 knockout males and knockout females of both lines had LH levels that were not statistically significantly different from WT (Fig. 6, A and B, and Table 1). Gonadotropin levels did not differ significantly between WT and heterozygous mice.

Administration of a peptide that consists of the C-terminal 10 amino acids of metastin is a powerful stimulus for GnRH-
induced gonadotropin secretion in many species (as reviewed in Ref. 15). Animals were injected sc with 50 nmol C-terminally amidated mouse kisspeptin 110–119 (metastin 43–52) and killed 30 min after injection. As expected, gonadotropin levels did not rise in male or female Gpr54 knockout animals. The gonadotropin responses in Kiss1 knockout animals were comparable to those of WT and heterozygous animals (Fig. 6, A–D, and Table 1). The subgroup of Kiss1 knockout females with higher gonadal weights (open circles in Fig. 6) had less robust gonadotropin responses to exogenous kisspeptin 110–119 than Kiss1 knockout females with lower gonadal weights.

**Testosterone levels and sperm production**

Baseline serum testosterone levels were significantly lower in Gpr54 knockout male mice but not in Kiss1 knockout males in comparison with WT (Kiss1−/− 91 ± 61 ng/dl, nonsignificant; Gpr54−/− 19 ± 22 ng/dl, P < 0.05; WT 155 ± 176; Fig. 7A and Table 1). The sperm count of both Kiss1 and Gpr54 knockout mice was reduced compared with WT (Fig. 7B). However, considerable phenotypic variation was observed because some knockout animals of both lines had no sperm, whereas others had sperm counts approaching those of WT animals.

**Fertility**

Five Kiss1 and five Gpr54 knockout males were mated with WT females of proven fertility for 11–36 wk, but no pregnancies were observed. Similarly, no pregnancies have been observed when Kiss1 and Gpr54 knockout females (five each) were mated with WT males of proven fertility.

**Discussion**

In this report, we demonstrate abnormal sexual development in mice with a targeted deletion of Kiss1, whose protein product is proteolytically processed to generate ligands for Gpr54, and contrast these findings with those of Gpr54 knockout mice generated in the same genetic context. Both Kiss1 and Gpr54 knockout mice have impaired sexual maturation, low gonadotropin levels, and abnormal gametogenesis as documented by these studies and others (16).

The endocrinological phenotypes of the Kiss1 and Gpr54 knockout mice were consistent with hypogonadotropism. When mouse metastin 43–52 was injected sc, all genotypes and genders (except Gpr54 null mice) responded with a significant increase in LH and FSH. In several previous studies, kisspeptin/metastin has been found to be a robust stimulus for GnRH release from the hypothalamus (reviewed in Ref. 15). In the absence of any other data, we assume that the robust response to exogenous metastin in Kiss1 knockout mice is due to the same mechanism of triggering GnRH and, by extension, LH release. Additional studies will determine whether a direct effect of metastin at the pituitary, where Gpr54 is expressed, can be uncovered using this model.

Pregnancies were not observed when either Kiss1 or Gpr54 knockout mice were housed with WT mice of proven fertility. The infertility of these mice could be multifactorial including 1) lack of preputial separation in males impeding intercourse, 2) lack of estrous cycling in females, 3) changes in mating behavior in both genders, although this was not formally assessed in this study, and 4) the proposed role of kisspeptin/Gpr54 system in placentation (17, 18). In vitro fertilization using sperm from null males would determine whether sperm, when present, are capable of fertilization.

Males of both knockout lines exhibited a slightly decreased anogenital distance at the time of weaning, although this difference did not reach statistical significance. This suggests that kisspeptin/Gpr54 may function during fetal and/or juvenile development, a possibility raised by a male patient carrying mutations in GPR54 who exhibited microphallus and cryptorchidism (19). After weaning, the difference in anogenital distance between knockout and WT males became marked; similar patterns of anogenital distance have been observed in LH receptor knockout mice (11). Anogenital distance at this age primarily reflects the action of testosterone (11), although influences from the fetal and juvenile time windows may contribute to a total cumulative effect. Along with the failure of preputial separation to develop in most of the knockout males, the postweaning difference in anogenital distance further suggests a failure of sexual maturation.
Importantly, considerable phenotypic variability was apparent in the Kiss1 knockout females. One subgroup had a more severe phenotype closely resembling that of Gpr54 knockout female mice, with small vaginal openings, small ovaries, and scant folliculogenesis. When given kisspeptin, this subgroup of mice responded with the largest increments in LH and FSH, possibly due to the lack of negative feedback by estrogen.

The other subgroup of Kiss1 knockout mice had larger gonadal weights, larger vaginal openings, and vaginal smears showing persistent vaginal cornification. This pattern has been observed in senescent mice and can also be induced by administration of high-dose estradiol in the neonatal period (20, 21). Although blood volume requirements precluded direct assay of estradiol in these animals, the large uterine weight, the persistent vaginal cornification, and the blunted response to kisspeptin suggested the presence of higher estrogen levels. Although we first thought that this bimodal pattern belonged only to the Kiss1 group, we did find one Gpr54 knockout female with persistent vaginal cornification and a large uterus. Although this is only one observation, we feel it may reflect specific mechanisms or thresholds required for pubertal development.

Kiss1 knockout males have a more modest phenotype than Gpr54 knockout males. The testes of Kiss1 null males are significantly larger than those of Gpr54 null males probably explaining the differences in serum testosterone, body weight, and sperm count. Basal gonadotropins are also slightly higher in Kiss1 knockout males than in Gpr54 knockout males, although this is not statistically significant. Although the Kiss1 null male data are not obviously bimodal, Kiss1 knockout female data, additional studies are needed to determine whether real heterogeneity exists within Kiss1 and Gpr54 knockout male mice.

Although the hypogonadism of Kiss1 knockout mice is consistent with the absence of a major compensatory ligand or constitutive activity of Gpr54, the phenotypic variability observed in Kiss1 knockout female mice does raise several important physiological possibilities: 1) another currently unknown ligand that can weakly stimulate Gpr54, 2) a previously unappreciated inhibitory role for kisspeptin, 3) modest constitutive activity of the Gpr54 receptor not previously detected but known to exist for many G protein-coupled receptors (22), 4) minute amounts of functional Kiss1 transcript and kisspeptin below the limits of detection of the assays employed in this study, 5) intrauterine position and possible steroid or kisspeptin transfer via amniotic fluid (23) or from the mother, and 6) genetic polymorphisms, although we failed to identify any founder or clear inheritance pattern in our colony of Kiss1 null mice. Although there are clear
differences between rodent and human reproduction, it is interesting to note that some patients harboring mutations in GPR54 do not have complete hypogonadotropic hypogonadism but rather exhibit partial pubertal development, low-amplitude LH pulsations, and increased (as opposed to decreased) responsiveness to exogenous GnRH (2, 24).

Gpr54 female mice, when stimulated with exogenous estrogen, can produce an LH surge. However, this surge is blocked when the animals are pretreated with acyline, suggesting the presence of a Gpr54-independent pathway capable of stimulating GnRH secretion (25). Collectively, these observations suggest that the kisspeptin/Gpr54 system is a key regulator of GnRH release but is not strictly required for GnRH secretion.

In general, analogous phenotypes are seen between ligand and receptor knockouts across the hypothalamic-pituitary-gonadal axis. For example, the LH receptor knockout mouse (26, 27) is remarkably comparable to the LHβ null model (28), indicating that cross-talk with other structurally comparable ligand-receptor pairs is unlikely. The FSH receptor knockout mouse (29, 30) and the FSHβ knockout mouse (31) both exhibit sterility in females and oligospermia and reduced fertility in males. Because kisspeptin modulates GnRH secretion, our targeted deletions of Kiss1 and Gpr54 are best compared with hpg and Gnrhr mutant mice. The hpg mice harbor a 33.5-kb deletion that results in a null mutation of the Gnrh gene. Spermatogenesis in hpg mice is arrested at the diplotene stage, and folliculogenesis rarely proceeds beyond the preantral stage (32). GnRH receptor mutant mice, recently created by N-ethyl-N-nitrosourea mutagenesis, also demonstrate a meiotic block of spermatogenesis, but folliculogenesis proceeds until the early antral stage (33). Although precise comparison between the hpg and Gnrhr mutant mice and our models is not possible due to strain differences, hpg mice have several features suggesting a more severe phenotype, including smaller ovarian size, earlier arrest of folliculogenesis, atretic interstitial testicular tissue, and undetectable FSH levels, and thus providing further support to the concept that the kisspeptin/Gpr54 system is not strictly required for GnRH secretion.

In summary, the phenotypes of Kiss1 and Gpr54 knockout mice are directionally parallel, with both sets of mice demonstrating hypogonadotropism. However, the degree of severity of the two sets of knockout mice are different with...
Kiss1 knockout mice being less severely affected than their receptor counterparts. In particular, the Kiss1 knockout females have a bimodal phenotype, with about 50% of the females having larger gonadal weight and persistent vaginal cornification. Although the reason(s) underlying this variability in phenotype are not known, these observations suggest the presence of novel biological thresholds for the initiation of sexual maturation, acting either alone or in concert with modest GnRH secretion.

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