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Role of Fibroblast Growth Factor Signaling in Gonadotropin-Releasing Hormone Neuronal System Development

Wilson C.J. Chung and Pei-San Tsai

Department of Integrative Physiology and Center for Neuroscience, University of Colorado at Boulder, 114 Clare Small, Boulder, CO 80309-0354 (USA), Tel. +1 303 735 1877, Fax +1 303 492 0811

Pei-San Tsai: pei-san.tsai@colorado.edu

Abstract

There is growing evidence demonstrating that fibroblast growth factor (FGF) signaling is important for the development of the gonadotropin-releasing hormone (GnRH) neuronal system. In humans, loss-of-function mutations in FGF receptor 1 (*Fgfr1*) and *Fgf8* lead to hypogonadotropic hypogonadism (HH) with or without anosmia. Insights into how FGF signaling deficiency disrupts the GnRH system in humans are beginning to emerge from studies using transgenic mouse models. In this review, we summarize GnRH system defects in several lines of FGF signaling-deficient mice. We showed that FGF signaling is critically required for olfactory placode induction, differentiation, and GnRH neuronal fate specification and postnatal maintenance. Extrapolating from these transgenic mouse data, we suggest that idiopathic HH in patients harboring loss-of-function *Fgfr1* and/or *Fgf8* mutations is not merely a result of defective GnRH neuronal migration, but also insults accumulated in the GnRH system during fate specification and postnatal development.

Gonadotropin-releasing hormone (GnRH) neurons are central to reproductive function in all vertebrates. Unlike most neurons in the brain, GnRH neurons are not born within the central nervous system (CNS) [1, 2]. Although the origin of GnRH neurons in non-mammalian species is still under debate [3, 4], the consensus in mammals is that GnRH neurons emerge from the olfactory placode during early embryogenesis and subsequently migrate across the cribriform plate to enter the forebrain. Once migration ceases, GnRH neurons target their axons to the external zone of the median eminence (ME) for hormone release [5]. Thus, the formation of the GnRH system during development requires the entrance of GnRH neurons into three distinct developmental phases including fate specification, migration, and axon targeting. Since the disruption of any of these developmental phases could lead to an aberrantly formed GnRH system and reproductive impairment, each developmental phase must be intricately regulated.

In 1995, we identified fibroblast growth factors (FGFs) as potent neurotrophic regulators of immortalized GT1 GnRH neuronal cell lines [6] and hypothesized that the disruption of FGF signaling could be an underlying cause for human reproductive impairment. This prediction later came true when FGF receptor 1 (*Fgfr1*) was identified as a causal gene underlying an autosomal dominant form of Kallmann syndrome (KS) [7], a human disorder characterized by hypogonadotropic hypogonadism (HH) and anosmia. A few years later, Pitteloud et al. [8, 9] showed that loss-of-function *Fgfr1* mutations also caused idiopathic HH (IHH) in normosmic individuals, reaffirming a strong relationship between FGF signaling and function of the GnRH system. Most recently, Falardeau et al. [10] demonstrated that mutations in one of the FGF ligands, *Fgf8*, could underlie varying degrees of IHH with or without anosmia. Of note, although these human studies revealed an important link between FGF signaling and function of the GnRH system, it is unclear whether FGF signaling

deficiency alters genesis, migration, or axon targeting of developing GnRH neurons, or if they lead to a postnatal dysfunction of the GnRH system. At the mechanistic level, these questions are very difficult to answer in humans. To circumvent this problem, investigations using mouse models deficient in various components of FGF signaling have been launched.

The development of GnRH neurons in mice has been extensively characterized. The olfactory placodes in mice develop from the anterior neural ridge [11] as the ventrolateral ectodermal thickenings. At embryonic day 10 (E10), the placode invaginates to form the olfactory pit, which invaginates again to form a secondary medial recess after 12 h. GnRH neurons (defined by the production of GnRH) are first seen in the medial olfactory pit at about E11.0–E11.5 [1, 12]. After fate specification, GnRH neurons begin an en masse migration at E11.5 to enter the forebrain. The migration of GnRH neurons in the nasal cavity is guided by the peripherin-positive tracts of the vomeronasal (VNN) and olfactory nerve [13]. By E14.5, the majority of GnRH neurons have crossed the nasal-brain junction. After reaching the forebrain, GnRH neurons make a ventral turn to follow a subset of peripherin-positive fibers to reach their final destinations [14]. At this time, a portion of GnRH neurons begins to extend their axons to the ME [15–17]. By E16, a network of GnRH neurons can be observed in a bilateral continuum from the base of the olfactory bulbs to the ME, and the formation of the GnRH neuronal system is largely complete.

To date, a long list of transcription factors, guidance molecules, neurotransmitters, cell surface molecules, growth factors, and receptors have been shown to regulate distinct phases of GnRH neuronal development in the mouse [18, 19]. This list speaks to the impressive redundancy involved in the formation of a critical neuroendocrine system. The pressing questions are, then, which regulators are (1) truly indispensable and (2) bear significant clinical relevance to human GnRH system defects? Compelling evidence now places FGF signaling as a prime candidate that is both indispensable and clinically relevant to the formation and maintenance of GnRH neurons. The goal of the present review is to provide an overview of GnRH system defects in transgenic mice deficient in various components of FGF signaling. By doing so, we will answer two important questions. First, which FGF ligand(s) and receptor(s) critically regulate GnRH neuronal development? Second, which phase(s) of GnRH neuronal development (fate specification, migration, or axon targeting) is severely impacted by FGF signaling deficiency? Extrapolating from the mouse studies, we hope to shed light on how FGF signaling components interact in a time-dependent fashion to support the development and maintenance of the human GnRH system.

Transgenic Mouse Models

This review will analyze GnRH system defects in four lines of transgenic mice deficient in FGF signaling. Each transgenic mouse line provides a unique tool for disrupting FGF signaling, but also has its own limitations (table 1).

A transgenic mouse (dnFGFR mouse) was generated by targeting the expression of a dominant-negative FGFR (dnFGFR) to GnRH neurons using the rat GnRH promoter [20]. Although this mouse allows the tissue-specific disruption of FGFR function in GnRH neurons, it comes with two limitations. First, it is not suitable for studying the very early phase of GnRH neuronal development (including fate specification) because GnRH promoter activity at this early period is presumably too weak to drive the expression of dnFGFR. Second, since dnFGFR disrupts the function of FGFRs 1, 2, and 3 [20], the observed phenotypes cannot be attributed to the disruption of a specific FGFR subtype. To circumvent these limitations, we utilize mice that are hypomorphic for *Fgfr1* (*Fgfr1* hypomorphs) [21] and knockout for *Fgfr3* (*Fgfr3* KO) [22] to elucidate the contribution of each receptor and to ensure their deficiency/deletion at the very beginning of GnRH neuron

genesis. The reasons for concentrating on these two FGFRs are described in the section below. Since FGF8 has been identified as an FGF ligand indispensable for the morphogenesis of the olfactory region [23], the birthplace of GnRH neurons, we also use mice hypomorphic for *Fgf8* (*Fgf8* hypomorphs) to examine the specific contribution of this ligand. It is important to note that *Fgfr1* hypomorphs, *Fgf8* hypomorphs, and *Fgfr3* KO bear the global deletion/deficiency in FGF signaling components; thus, indirect effects upon GnRH neurons cannot be ruled out. However, compared to mice with tissue-specific disruption, the hemizygous state of global disruption in these mice may be more comparable to humans harboring *Fgfr1* and/or *Fgf8* mutations.

Regulation of Gonadotropin-Releasing Hormone Neuronal Fate Specification by Fibroblast Growth Factor Signaling

Landmark studies showed that the vast majority (~80%) of mouse GnRH neurons undergo their last division in the olfactory placode between E9.5 and E10.5 [2]. Interestingly, these postmitotic olfactory placode progenitor cells do not express detectable levels of GnRH mRNA or peptide prior to E11.5 [1, 2]. In this sense, one can define the period of GnRH neuron fate specification to occur between E9.5–E11.5. The absence of measurable GnRH peptide prior to E11.5 is a major hurdle for studying the molecular processes that regulate GnRH neuronal fate specification, because at present the only marker for GnRH neurons is GnRH itself. Therefore, postmitotic olfactory placode progenitor cells that are destined to become GnRH neurons cannot be identified during a large portion of the fate specification, before they express measurable GnRH levels. Notwithstanding this major caveat, our laboratory has accumulated mounting evidence that the activation of FGF signaling is important for the fate specification of the mouse GnRH neuronal system.

In mice, FGF signaling is mediated by four specific types of FGFRs [24]. Localization studies from our laboratory showed that the E10.5 mouse olfactory placode contained abundant levels of FGFR1, FGFR2 and FGFR3, but not FGFR4 protein [25]. Furthermore, dual immunocytochemistry detected the presence of FGFR1 and FGFR3, but not FGFR2 protein in E15.5 and postnatal-day-3 GnRH neurons [25]. This is consistent with an earlier observation showing that *Fgfr1* and *Fgfr3* mRNA is expressed in immortalized mouse GnRH neuronal cell lines [6]. The strongest data indicating that FGF signaling plays a role in GnRH neuronal fate specification are derived from in vitro nasal explants isolated from E10.5 embryos. In this experimental paradigm, the number of GnRH neurons in the nasal explants increased after 2 or 3 days in vitro [25]. This increase in GnRH neurons was inhibited by the FGFR antagonist SU5402, indicating the activation of FGFR is required for GnRH neuron fate specification [25].

The aforementioned studies provided the impetus for identifying the specific FGF signaling component(s) required for GnRH neuronal fate specification. Based on the presence of FGFR1 and FGFR3 in GnRH neurons, we hypothesized that activation of these receptors was critical for mouse GnRH neuronal fate specification. Therefore, disruption of FGFR1 or FGFR3 function is expected to result in a defective GnRH neuronal system. We tested this hypothesis using mice globally deficient in FGFR1 (*Fgfr1* hypomorphs) or FGFR3 (*Fgfr3* KO), which have a major advantage. Unlikely tissue-specific KOs that require the GnRH promoter to be active before disruption can occur, the global disruption of genes guarantees FGFR1 or FGFR3 deficiency at the time of GnRH neuronal fate specification. We first examined the total number of GnRH neurons in the entire heads of newborn wild-type, heterozygous and homozygous *Fgfr1* hypomorphs, and found that the number of GnRH neurons in homozygous *Fgfr1* hypomorphs was about 88% lower than their wild-type littermates [26]. Furthermore, there was no significant difference between heterozygous *Fgfr1* hypomorphs and their wild-type littermates [26]. On the other hand, the number of

GnRH neurons was unaffected in newborn *Fgfr3* KO mice [26]. These whole animal studies corroborated the nasal explant studies and supported the idea that activation of FGFR function may be a key pathway required for the emergence of GnRH neurons [25]. Furthermore, by comparing the GnRH neuronal system between *Fgfr1* hypomorphic and *Fgfr3* KO mice, we established that the activation of FGFR1, but not FGFR3, is required for the normal number of GnRH neurons in the newborn mouse head.

Of the 22 identified FGF ligands, there are several lines of experimentally derived evidence indicating that FGF8 is the most likely FGF candidate to be involved in the early development of GnRH neurons. First, *Fgf8* mRNA is found in the ectodermal region ventral/lateral of the telencephalic commissural plate on E9.5, which will form the olfactory placode [23, 27]. This expression profile suggests that FGF8 signaling is critically important for the induction and differentiation of the mouse olfactory placode. Second, a partial loss of FGF8 function resulted in the absence of the olfactory bulbs and vomeronasal organs (VNO) [26, 28]. The absence of the VNO has a special relevance given that the VNO also originates in the olfactory placode [2]. Third, FGF8 has been shown to bind and readily activate FGFR signaling, including FGFR1, in cell lines [10]. Therefore, we hypothesized that disruption of FGF8 signaling will severely impair the genesis of the GnRH neuronal system. To test this hypothesis, we examined the GnRH neuronal system in newborn *Fgf8* hypomorphic mice, which in the homozygous state shows a ~54% downregulation of *Fgf8* mRNA [28]. Much to our surprise, the GnRH neuronal system in homozygous *Fgf8* hypomorphs was not partially affected, but eliminated in its entirety [10, 26]. An equally striking observation was that heterozygous *Fgf8* hypomorphs had approximately 50% fewer GnRH neurons remaining as compared to wild-type littermates [10, 26]. These results led to our conclusion that the prenatal development of the mouse GnRH neuronal system is extremely dependent on the presence of FGFR1 and FGF8 signaling.

A major constraint of studies in newborn mice mentioned before is that they do not elucidate whether FGF8 signaling is required for GnRH neuronal fate specification or other developmental processes, such as neuronal survival after emergence. Therefore, we expanded our studies to include the analysis of the E11.5 GnRH neuronal system, when GnRH neurons are first detectable using immunocytochemistry. By examining the emergence of GnRH neurons from the E11.5 olfactory placode, we were able to directly answer the question: Is FGF8 signaling required for the fate specification process of GnRH neurons in the olfactory placode? In contrast to wild-type embryos, we did not detect any GnRH neurons in the olfactory placode of homozygous *Fgf8* hypomorphs on E11.5 or in any subsequent time points that were examined [26]. Together with the previously described nasal explant studies, these data strongly suggest that FGF8 signaling acting through FGFR1 is critical for GnRH neuron fate specification in the mouse olfactory placode.

Interestingly, we found that the secondary recess of the E11.5 olfactory placode from where GnRH neurons emerge is virtually absent in homozygous *Fgf8* hypomorphs. The dysgenesis of this secondary recess is likely the underlying cause for the failure of GnRH neurons to emerge (fig. 1). Our preliminary data showing that the VNO is missing in the homozygous *Fgf8* hypomorphs are consistent with the notion that this recess fails to develop and give rise to differentiated derivatives. Our data also suggest the presence of discrete developmental zones in the olfactory placode [13], with FGF8 signaling deficiency more likely to impact the zone that gives rise to the epithelial cells of the VNO and GnRH neurons. Currently, we are studying how a partial loss of FGF8 function could severely disrupt the developmental organization of the secondary recess of the olfactory placode.

Regulation of Gonadotropin-Releasing Hormone Neuron Migration by Fibroblast Growth Factor Signaling

The organization and functionality of the GnRH neuronal system depends not only on the proper emergence of GnRH neurons from the olfactory placode, but also on the accurate migration from the olfactory placode to the preoptic/hypothalamus region. Indeed, the failure of GnRH neurons to migrate and transition from the nasal cavity into the anterior forebrain was considered to be the major cause of GnRH deficiency in KS [29]. This view was based on the observation that GnRH migration was arrested just prior to entering the forebrain in a 19-week-old fetus with the X-linked form of KS [30]. Indeed, no GnRH neurons were detected in the forebrain regions where they were expected during this stage of embryonic development, but were instead found beneath the forebrain within the meninges [29]. Moreover, the VNN fibers in this KS fetus also ended within the meninges and failed to contact the developing olfactory bulbs [29].

GnRH migratory defect is frequently extrapolated to be the key cause of IHH in all forms of KS, including KS patients who harbor loss-of-function mutations in the *Fgfr1* or *Fgf8* genes [7, 10]. It is possible that GnRH migration is affected in these KS patients, but there are no studies verifying this in human fetuses with mutations in *Fgfr1* or *Fgf8*. In fact, mouse studies done in our laboratory found little evidence supporting the view that defects in FGF signaling cause impaired GnRH neuronal migration. Only a slight arrest (<10%) in GnRH neurons was detected in the intra-hemispheric area of newborn dnFGFR mice as compared to their wild-type counterparts [31]. This slight arrest in GnRH neuron migration is inconsistent with the significant reduction of GnRH neurons in the preoptic/hypothalamus of adult dnFGFR mice. The majority of GnRH neurons in dnFGFR mice still migrated normally into the brain [20], and the loss of GnRH neurons in dnFGFR mice is due primarily to postnatal disappearance instead of migratory defects. These data suggest that the disruption of FGFR function directly at the level of GnRH neurons plays only a minor role in their migration [31].

One might also question if global deficiency of FGF signaling could alter the path-finding of axons that guide GnRH neuronal migration, thereby indirectly disrupting this migratory process. It is interesting to note that the peripherin-positive fibers of VNN were in fact disrupted and failed to target the telencephalon of homozygous *Fgf8* hypomorphs (fig. 1) [26]. However, it is important to emphasize that GnRH neurons were already missing in these transgenic mice prior to migration; thus, the cause of GnRH system defect in these mice is failure to emerge, not failure to migrate. In heterozygous *Fgf8* hypomorphs with 50% of GnRH neurons still remaining, the reduction in GnRH neurons was also not due to defective GnRH migration. We base this conclusion on our analysis of the entire head of these newborn mice, in which we did not find any evidence of abnormal accumulation of GnRH neurons along the migrational pathway prior to entering the brain [26]. A similar observation was made for homozygous *Fgfr1* hypomorphs. Consequently, data from mice suggest that IHH in KS patients harboring *Fgfr1* or *Fgf8* mutations is more likely caused by defects in GnRH neuronal fate specification than in migration.

Regulation of Gonadotropin-Releasing Hormone Axon Targeting by Fibroblast Growth Factor Signaling

During axon targeting, GnRH fibers extend ventrally in a highly directional manner to terminate upon the fenestrated capillaries within the external zone of the ME. GnRH is released into the primary capillary plexus to reach the hypothalamo-pituitary portal veins, and ultimately the anterior pituitary. Thus, this is the stage that confers final fertility. In

order for GnRH axons to target the ME properly, two events must occur: (1) the extension of the axons, and (2) the directional growth of axons to the proper target. There is virtually nothing known about the mechanisms of GnRH axon targeting in humans. However, there is a wealth of information on how FGF signaling regulates GnRH axon targeting in mice.

Regarding the extension of axons, *in vitro* data from our laboratory showed that this process is regulated by FGF signaling [25]. The addition of FGF2, a prototypic FGF, significantly stimulated neurite outgrowth in cultured E15.5 GnRH neurons [25]. Importantly, GnRH neurons isolated from dnFGFR mouse embryos exhibited attenuated neurite outgrowth in culture [32], suggesting FGF signaling is an endogenous cue that maintains GnRH axon growth. Regarding the directional axonal growth to the proper target, Silverman et al. [33] showed that GnRH neurons grafted into the third ventricle targeted axons to the ME, and this directional axonal growth was due to the presence of a diffusible chemoattractive signal(s) in the target [34]. To date, the identity of the diffusible chemoattractant(s) remains elusive, but several lines of evidence suggest FGFs might be one of the components. FGF2, a molecule produced in abundance by both endothelial cells and ventricular tanycytes of the ME, served as a diffusible cue to attract the directional growth of GnRH axons in an organotypic culture system [35]. The caveat is that while the study showed an attractive response of GnRH axons to an exogenously added FGF, it did not verify the role of FGFs as endogenous regulators in this process. To satisfy the criterion of FGF signaling as an endogenous chemoattractive regulator, we have demonstrated that in cultured embryonic GnRH neurons, the directional outgrowth of neurites toward the cocultured ME explant is significantly reduced in GnRH neurons expressing dnFGFR [32]. Further indirect evidence arises from the recent observation that anosmin-1, the diffusible extracellular product of *KAL1*, in turn exerts a modulatory role of FGF2/FGFR1 signaling [36].

Overall, our *in vitro* results showed attenuated FGF signaling disrupted the ability of GnRH neurites to extend and to grow in a directional manner. Do these *in vitro* alterations translate into GnRH system defects in the whole animal? There is evidence that suggests they do, but these *in vivo* defects are somewhat less striking. GnRH neurons in dnFGFR mice showed a significant but small reduction (15%) in the uptake of peripherally injected fluorogold, a retrograde tracer, indicating compromised contact between GnRH axons and circumventricular organs such as the ME [32]. That GnRH axon targeting is only modestly disrupted in dnFGFR mice argues for the presence of other compensatory mechanisms guiding GnRH axons *in vivo*. Since GnRH axon targeting is a highly axophilic event in which GnRH axons travel with other growing axons [34], these guiding axons could drive the extension and directional growth of GnRH axons via physical or other FGF-independent means. Overall, despite significant defects observed *in vitro*, adequate GnRH fibers have targeted the ME of dnFGFR mice to support reproduction. Current evidence suggests that GnRH axon targeting in the whole animal does not appear to be a process that critically requires FGF signaling.

Regulation of Postnatal Gonadotropin-Releasing Hormone System Function by Fibroblast Growth Factor Signaling

The proper timing of pubertal onset and reproductive senescence is key to a normal reproductive lifespan. In this regard, a healthy GnRH system becomes activated in a timely fashion and remains functional for a period of time optimal for the propagation of offspring. At present, there is direct and circumstantial evidence for a role of FGF signaling in the onset and termination of the mouse GnRH system function, thereby determining the duration of the reproductive lifespan.

Mouse GnRH neurons undergo dynamic remodeling in their morphology after birth, and this remodeling could have a functional significance [37]. During the pubertal transition, mouse GnRH neurons transform from having a complex dendritic tree bearing a few spines to having a simple dendritic structure bearing a large number of spines [37]. Consequently, the remodeled GnRH neurons in adults possess high dendritic spine densities, indicating the acquisition of abundant excitatory synaptic inputs [38] that could drive puberty. These observations contradict the previous reports that GnRH neurons receive sparse synaptic inputs [39, 40] and demonstrate pubertal onset is accompanied by the morphological maturation of GnRH neurons. Could FGF signaling be involved in the morphological maturation of postnatal GnRH neurons? Results from our laboratory suggest this is likely. We have shown that FGF signaling stimulates in vitro neurite branching of postnatal, but not embryonic, GnRH neurons [25]. Although the significance of this finding is unclear, it implicates FGF signaling in the postnatal alterations of GnRH neuronal morphology. Of further importance, dnFGFR female mice exhibit delayed puberty [20]. It stands to reason that delayed puberty in dnFGFR mice may be correlated with the reduced ability of their GnRH neurons to undergo morphological remodeling conducive to the receipt of excitatory inputs.

In addition to the postnatal maturation of GnRH neuronal morphology, FGF signaling could be required to maintain the postnatal GnRH system, thus promoting a healthy reproductive lifespan. Wu et al. [41] reported that there were ca. 1,100 GnRH neurons in neonatal mice, and this number declined to ca. 800 in adult mice. From then on, the number of GnRH neurons remained either unchanged [42] or declined only modestly by 18% in aging mice [43]. Overall, there does not appear to be a marked decrease in GnRH neurons with aging, suggesting GnRH neurons are unlikely to undergo dedifferentiation or cell death as they age. Rather, the aging GnRH system is more likely to undergo functional declines in GnRH synthesis and secretion [44]. Contrary to these observations, we show that postnatal dnFGFR mice, as they age, suffer a 70% loss in GnRH neurons and 95% loss in hypothalamic GnRH peptide concentration, respectively [26]. Our data suggest neurotrophic support by FGF signaling is absolutely critical for the maintenance of the postnatal GnRH system. In this sense, GnRH neurons are not unlike other neuronal systems such as motor neurons that rely heavily upon neurotrophic support for postnatal survival [45, 46]. Future studies are needed to examine the time course of postnatal GnRH neuronal loss and the mechanism underlying this loss.

Clinical Implications

Defective GnRH neuron migration was thought to be one of the major causes of IHH in all forms of KS. The lack of gross defects in GnRH neuronal migration in our dnFGFR, *Fgfr1* hypomorphic and *Fgf8* hypomorphic mice suggests that this extrapolation may not hold true for KS patients harboring loss-of-function mutations in *Fgfr1* and/or *Fgf8*. On the other hand, we have evidence indicating that disruption of FGFR1 and FGF8 signaling may have caused major defects during the fate specification phase of the GnRH system. Furthermore, our studies showed that FGF signaling is required for the postnatal maintenance and functionality of the GnRH neuronal system. Altogether, a single mutated copy of the *Fgfr1* and/or *Fgf8* gene may lead to multiple insults on the GnRH neuronal system during prenatal and postnatal life.

Studies from mouse clearly showed that FGF signaling is involved, albeit not of equal importance, in all phases of GnRH neuronal development, maintenance, and functionality. For example, the global disruption of FGF signaling impacts primarily the fate specification phase of the GnRH neuronal system, whereas we did not observe gross abnormalities in GnRH neuronal migration. One might argue that if GnRH neurons were able to emerge,

FGF signaling deficiency may still cause major migratory impairment later on. Our data from dnFGFR do not fully support this notion. In dnFGFR mice in which the tissue-specific disruption of FGFR occurs only after fate specification, GnRH neurons emerged normally, as expected (unpubl. data). After the emergence of these neurons, we observed only a slight arrest in GnRH neuronal migration [31]. The major GnRH system defect in these mice appears to be the precipitous postnatal loss of GnRH neurons that likely led to premature reproductive senescence. Based on these observations, we hypothesize that a heterozygous *Fgfr1* and/or *Fgf8* mutation in KS patients may lead to multiple insults on the GnRH neuronal system during fate specification and after birth. The postnatal loss of GnRH neurons may, in part, explain the manifestation of adult-onset IHH seen in a patient harboring *Fgf8* mutation [10].

Clinical observations showed that carrying a single identical gene mutation within and across IHH families is not predictive of the clinical reproductive and non-reproductive phenotypes, suggesting incomplete penetrance of a specific gene defect [47]. Another interesting and novel explanation for the lack of predictive precision could be that the severity of IHH depends on the number of gene defects found in a patient [47]. Indeed, Pitteloud et al. [47] identified a KS patient harboring heterozygous defects in both *Fgfr1* and *Nelf*. Recently, compound defects in *Fgfr1* and *Fgf8* have also been identified in two cases with normosmic IHH [10]. Although these invaluable clinical observations reveal the genetic complexity of IHH and KS, it is not possible to study directly in humans how these multiple mutations exacerbate IHH in KS patients. In theory, the mouse model could be extremely useful for testing the compound effects of such gene defects. For example, by crossbreeding *Fgfr1* hypomorphs with *Fgf8* hypomorphs, we can examine whether heterozygous defects on multiple IHH or KS-associated genes have compounding effects on the GnRH neuronal system.

KS patients represent a unique ‘human model’ that provides insights into signaling factors involved in the organization of a critical neuroendocrine system. Further elaboration on the molecular mechanisms induced by these factors is accomplished by studying transgenic mice. Using the mouse models, we have shown that FGF signaling is required not only for olfactory placode induction and differentiation, but also for fate specification and postnatal maintenance of the GnRH neuronal system. In sum, we speculate that IHH in KS is not merely a result of defective GnRH neuronal migration, but also insults accumulated in the GnRH system during pre- and postnatal development.

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Fig. 1.

Parasagittal schematic representation of the mouse olfactory placode (red) on E11.5. The secondary recess (arrow) in the olfactory placode, from where the GnRH neurons (blue) emerge, is present in the wild-type, but not in the homozygous *Fgf8* hypomorph embryo. This suggests that the formation of this recess is FGF8 dependent. FGF8 signaling is also important for the development of the peripherin-positive fibers (green) of the VNN, which fail to enter the homozygous *Fgf8* hypomorph mouse forebrain. However, because GnRH neurons were eliminated prior to entering the migrational phase, we hypothesize that the primary impact of FGF8 deficiency is upon the fate specification process of GnRH neurons.

Table 1
List of transgenic mice deficient in FGF signaling used in our studies

Name of mouse	Deficiency syndrome	Utility of mouse model	Limitations of model
dnFGFR	Disrupts functions of FGFRs 1, 2 and 3 specifically in GnRH neurons	Study tissue-specific effects of attenuated FGF signaling on later phases of GnRH neuronal migration, axon targeting and postnatal function.	(1)Not appropriate for studying GnRH neuronal fate specification and early migration. (2)The extent of FGFR function disruption depends on the activity of the GnRH promoter. (3)Does not reveal which component of FGF signaling is involved.
<i>Fgfr3</i> KO	Global deletion of functional FGFR3 protein.	Study effects of global deletion of FGFR3 on GnRH system development and postnatal function.	Not tissue specific, but appropriate for examining <i>all</i> stages of pre-/post- natal GnRH system development.
<i>Fgf8</i> hypomorph	Global reduction of functional FGF8 protein due to a splicing defect caused by neo-cassette insertion.	Study effects of global reduction of FGF8 on GnRH system development and postnatal function.	Not tissue specific, but appropriate for examining <i>all</i> stages of pre-/post- natal GnRH system development.
<i>Fgfr1</i> hypomorph	Global reduction in functional FGFR1 protein due to a splicing defect caused by neo-cassette insertion	Study effects of global reduction in FGFR1 on GnRH system development and postnatal function.	Not tissue specific, but appropriate for examining <i>all</i> stages of pre-/post- natal GnRH system development.