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GnRH, anosmia and hypogonadotropic hypogonadism - where are we?

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Abstract

Gonadotropin releasing hormone (GnRH) neurons originate in the nasal placode and migrate into the brain during prenatal development. Once within the brain, these cells become integral components of the hypothalamic-pituitary-gonadal axis, essential for reproductive function. Disruption of this system causes hypogonadotropic hypogonadism (HH). HH associated with anosmia is clinically defined as Kallman syndrome (KS). Recent work examining the developing nasal region has shed new light on cellular composition, cell interactions and molecular cues responsible for the development of this system in different species. This review discusses some developmental aspects, animal models and current advancements in our understanding of pathologies affecting GnRH. In addition we discuss how development of neural crest derivatives such as the glia of the olfactory system and craniofacial structures control GnRH development and reproductive function.

The GnRH neurons – Regulators of fertility

The hypothalamic-pituitary-gonadal (HPG) axis is considered to be an evolutionary innovation specific to vertebrates [1; 2]. The neuroendocrine gonadotropin releasing hormone (GnRH) neurons are integral components of this axis, regulating sexual development and reproductive function (see Figure 1). In mammals, the primary terminal field of the neurosecretory GnRH neurons is the median eminence. Here, the cleaved and amidated GnRH decapeptide is released into portal vessels where it is transported to the anterior pituitary gland. GnRH activates receptors on pituitary gonadotropes, triggering synthesis and release of the gonadotropins luteinizing hormone (LH) and follicle stimulating (FSH). LH and FSH are necessary for gonadal function. In the ovary, LH acts upon theca cells that produce the androgen substrate required for ovarian estrogen biosynthesis. In the

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testis, LH induces Leydig cells to produce testosterone. In both males and females, FSH stimulates maturation of germ cells. Thus, if GnRH release is compromised it translates into impaired reproductive maturation and function - e.g. hypogonadotropic hypogonadism (HH). HH can result from defects in GnRH neuronal development [3; 4; 5], GnRH synthesis [6], release [7; 8; 9; 10] or ligand/receptor (GnRH/GnRHR) pairing [4; 11; 12; 13].

Recent work has given us a better understanding of the cellular composition of the developing olfactory area, development of the GnRH cells and perturbations leading to HH. However, we still do not fully understand the cell types involved in GnRH neuronal development. This review discusses some of the similarities and differences of the GnRH system among animal models. In addition, we highlight some recent animal studies on GnRH neuronal embryonic lineage, craniofacial development and olfactory ensheathing cells that may explain the diversity of phenotypes observed in HH patients that resulted from a developmental perturbation.

GnRH Peptides

During early evolution, three paralogous GnRH genes (*gnrh1*, *gnrh2* and *gnrh3*) arose from two rounds of genome duplication [14]. Various isoforms of GnRH decapeptides have been found in all vertebrates: ranging from agnathans to mammals [15; 16; 17]. Characterization of non-mammalian vertebrates species including fishes, amphibians and reptiles identified up to 15 different GnRH variants [18; 19] that were classified into three main phylogenetic groups, [17; 20; 21; 22; 23; 24; 25], prior to gene identification, and historically named after the first class or species in which they were characterized [24; 26; 27; 28; 29; 30]. However, identification of the *gnrh* genes revealed a genetic twist - the *gnrh* genes expressed in vertebrates varies within teleosts as well as within mammals (Figure 2). All 3 GnRH genes are found in ancient teleosts including *medaka* [31; 32; 33]. However, genetic studies have shown that though multiple GnRH paralogs originated during evolution, the GnRH-3 family was lost in the tetrapod lineage [34]. In most teleosts, GnRH-3 is expressed by neurons of the terminal nerve/olfactory region and believed to function as a neuromodulator and to be indirectly linked to the reproductive neuroendocrine axis [31]. Observations made in sea bass indicate that GnRH-3 neuronal projections can innervate the retina and modulate retinal function [35]. *Gnrh2* (Human chromosome 20) is the most ancient form of GnRH. GnRH-2 peptide is often referred to as mesencephalic GnRH based on the anatomical location of the cells expressing the peptide. Studies in fish suggest that GnRH-2 might be a neuromodulator in the auditory system [31; 36; 37] or a melatonin-releasing factor in the pineal gland, participating in sleep/wake cycles [38]. However, during evolution, the preproGnRH-2 gene as well as the GnRH-2 receptor has been deleted or inactivated from the genome of many mammals [39]. Thus, a physiological role for GnRH-2 in mammals remains controversial. *Gnrh1* is present in most vertebrates (Human chromosome 8) but notably absent in modern teleosts including zebrafish [40; 41] [42; 43]. In modern teleosts, GnRH-3 adopted the role of GnRH-1 in reproduction [44]. GnRH-1 is the only form of the GnRH gene that exists in the rodent genome [45] (Summarized in Fig. 2). In mammals, GnRH-1 cells are located in the forebrain, distributed bilaterally on either side of midline. The actual location of GnRH-1 cells can vary rostrally to caudally depending on the species [46]. The pivotal function of the GnRH-1 peptide in controlling the HPG axis was proven in mice carrying a loss of function

mutation in GnRH-1 gene, hpg mice [47]. These mice exhibit deficient pituitary gonadotropin secretion and failure of gonadal postnatal development (either testes or ovary). In addition, GnRH-1 function in controlling the HPG axis was elegantly demonstrated by one of the very first examples of gene therapy/rescue experiment in animals. In 1986, A. J. Mason and coworkers showed that transgenic expression of GnRH-1 was sufficient to re-established reproductive competence in both hpg male and female mice [47; 48]. Proof of the same role for the GnRH-1 system in humans came from the identification of homozygous loss of function mutations in the GnRH1 gene in a family carrying normosmic HH [49].

Embryonic development of GnRH neurons

Deficits in the sense of smell and hypogonadism were first reported in 1856 by A. Maestre de San Juan and later, in 1944, by F. J. Kallmann [50]. Kallmann noticed a co-segregation of anosmia and hypogonadism in individuals from three families and suggested a hereditary nature of this syndrome, now commonly known as Kallmann syndrome (KS). The anatomical link between anosmia and hypogonadism would remain unknown for four decades.

Although genetic changes have added unexpected twists to understanding the hypophysiotropic GnRH system (for other reviews see [5; 14; 51; 52]) a striking similarity has been conserved throughout evolution in the development of the GnRH neurons responsible for controlling the HPG axis. In the late 1980s, developmental studies in mice, by two independent groups [53; 54; 55], revealed that GnRH-1 neurons migrate from the nose to the brain in association with developing sensory axons of the olfactory/vomerolateral terminal nerve system (see Figure 3). These studies found the crucial developmental link between the two main components affected in syndromic forms of HH (e.g. KS) [3]. Further characterization in mice indicated that the GnRH-1 cells migrated along a pathway [56] that is a subset of putative vomeronasal/terminal nerve fibers [56; 57]. GnRH-1 neurons have been subsequently described to originate in the developing nose and migrate along subsets of fibers to the brain in birds, amphibians, reptiles, medaka and mammals.

The Olfactory placode

Although GnRH-1 gene expression has been reported at morula and blastocyst stages, and transient expression is observed in both neuronal and non-neuronal cell types [5], birth dating studies in mice have shown that GnRH expressing neurons responsible for the HPG axis are born and fate specified around mid-gestation [53; 54; 63; 66; 67]. GnRH neurons form in a niche at the border between respiratory epithelium and vomeronasal/olfactory epithelium (Fig. 3 and 4A). Removal of the olfactory placode in amphibian and birds [68; 69], mouse models with lack olfactory placodal development [70] and laser ablation of GnRH cells from the nasal area in fish [71], support the notion that GnRH neurons with hypophysiotropic function arise in the olfactory pits, a structure that forms as the olfactory placode invaginates (summarized in Fig. 4B).

Tissue ablation of portions of ectoderm of the developing nose in chick and analysis of mice lacking AP-2 α , which delineates presumptive respiratory epithelia from olfactory epithelia [73], suggested that GnRH-1 neurons might originate from an area of the olfactory pit that

included the ectoderm responsible for the formation of the respiratory epithelium [72] (Figure 4C). Although ablation experiments (Fig. 4B,C) confirmed that the olfactory pit and putative respiratory epithelium were important for the differentiation of GnRH neurons, experiments of this kind could never fully rule out that: a) the analyzed GnRH cells originated somewhere else and then migrated and matured in the pit [74] or b) the ablated tissue (e.g. respiratory epithelium) was the source of necessary trophic factors needed for GnRH neuron differentiation or survival, rather than the site of origin of GnRH neurons themselves [72; 75; 76; 77] (Figure 4D). In fact, recent genetic tracing of Fgf8 producing cells of the respiratory epithelium indicated that these cells lacked neurogenic ability [78] (Figure 4E). These data are consistent with GnRH cells associated with the respiratory epithelium originating elsewhere, but does not address whether trophic factors within the respiratory epithelium are important for GnRH neuron differentiation or survival (see below).

Classically, the olfactory placode was believed to give rise to nonsensory respiratory, sensory olfactory epithelium, GnRH neurons and olfactory ensheathing cells and, unlike other sensory placodes, not to have a neural crest contribution [51; 79]. Recent cell and lineage tracings in chick and mouse confirmed that GnRH-1 neurons originate from cells that belong to the olfactory placode (Fig. 4F) [62; 80] and highlighted that the embryonic origin and/or molecular profile of the GnRH-1 neuronal progenitors within the placode is not homogeneous as previously believed [80]. In fact Forni and coworkers [80; 81] proposed that progenitor cells of putative neural crest origin and placodal origin contribute to the GnRH-1 neuron cell lineage. In addition, this work also proposed that that neural crest cells contributed to different cell populations in the olfactory epithelium (Figure 4F). Though multiple reports now support a neural crest contribution to the cells of the olfactory pit [82; 83; 84; 85] the embryonic heterogeneity of the GnRH-1 in mammals is still debated [62; 81; 86; 87; 88], perhaps exacerbated by controversies in teleosts.

Studies using a genetically modified Medaka fish model [64] showed that GnRH-3 and GnRH-1 neurons share the same olfactory embryonic origin and migratory path from nose to the brain, but did not address their lineage. Two recent studies using zebrafish expressing EGFP under the control of the GnRH-3 promoter obtained different results with respect to origin of GnRH-3 neurons. The first study showed that laser ablation of the soma of GnRH-3 neurons in the nasal area during early development resulted in a complete lack of olfactory, terminal nerve, preoptic area, and hypothalamic GnRH-3 neurons [40; 71]. At later developmental stages, these fish had arrested oocyte development and reduced average oocyte diameter [71], consistent with the lack of GnRH-3 neurons and loss of hypophysiotropic function. This study suggests that all GnRH-3 neurons originated from the olfactory region and migrate to the brain. However, a second study using a different transgenic zebrafish line concluded that the GnRH-3 system is actually formed by distinct GnRH-3 expressing neuronal populations that independently form in the olfactory region, hypothalamus, preoptic area, and trigeminal ganglion [77]. However, earlier studies in zebrafish [74; 77], based on lineage cell fate tracing, suggested that GnRH-3 neurons originated from developing neural crest [74; 89], similar to the GnRH-1 subpopulation recently detected in mouse [80].

Cranial morphogenesis and GnRH neurogenesis

The frontonasal mesenchyme adjacent to the developing olfactory placode is mainly composed of cells of neural crest origin that differentiate into cells of bone, cartilage and connective tissue of the nose [90]. The olfactory pit is the source of most of the migratory neurons, olfactory neurons and the respiratory epithelium of the nasal cavity. Molecular cross talk between the nasal mesenchyme and the olfactory pit are necessary for invagination of the olfactory pit, patterning of neurogenic areas and definition of precise milieus responsible for development of placodal derivatives, including GnRH-1 cells [91; 92; 93; 94]. Correct development of nasal cartilage, olfactory cavities and nasal connective tissue depends on molecular cues secreted by the invaginating placode [75; 95; 96; 97; 98]. Tipping the balance of cross talk between the nasal mesenchyme and the olfactory pit can have dramatic consequences, ultimately resulting in reproductive dysfunction as described below.

Trophic factors and craniofacial development

In the developing olfactory pit, the non-neurogenic respiratory epithelium is the main source of FGF8 (Figure 5A-C), a molecule that plays a key role in craniofacial development [78; 99]. FGF8 levels are crucial for midline facial integration and correct formation of nasal regions [75; 99; 100]. Mechanical excision of the respiratory epithelium and therefore the Fgf8 source, like loss of function of the *fgf8* gene, disrupts early stages in the formation of GnRH-1 neurons [76] (Fig. 4C, D). In humans and rodents, even partial loss of function in FGF8 signaling results in craniofacial defects, aberrant olfactory development and lack of GnRH-1 neurogenesis [75; 76; 101; 102; 103]. In humans [101], nonsense mutations in the *FGF8* gene have been linked with different penetrance and degrees of 1) cranial facial defects, including cleft lip and palate, osteoporosis, hearing loss, hypertelorism (abnormal distance between eye orbits), flat cleft palate and nasal bridge, and 2) gonadotropin-releasing deficiency and delayed puberty.

Recent work has shown how loss of FGF8 in the nasal region tips the balance between signals that subsequently alter the olfactory pit, GnRH-1 neurogenesis, and thus reproductive function [78] (Figure 5D-F). Areas that will give rise to neurons (neurogenic niches) in the olfactory placode are defined by pro-neurogenic signals such as Fgf8 and TGF- β factor antagonists (e.g. Noggin), in conjunction with neurogenic repressors signals such as Bone Morphogenic Protein-4 (BMP4) [104; 105]. Neurogenic repressors induce ectodermal cells to acquire epidermal cell fates [106]. Analysis of mutant mice with impaired *Fgf8* expression revealed an important relationship between dysmorphic growth of the nasal mesenchyme and neurogenesis in the olfactory pit (Fig. 5E, F). Neurogenic defects in the developing olfactory pit were a reflection of aberrant growth of the nasal mesenchyme and associated changes in morphogenic signals. Two independent studies showed that reduced Fgf8 expression translated into expansion of BMP4 expression areas in nasal mesenchyme [78; 99]. BMP signaling can trigger the expression of BMP antagonists, such as Nog, Chordin and Follistatin [107]. BMP antagonists play a key role in controlling olfactory neurogenesis [105; 108]. In line with this BMP4, was found to be a direct inducer of *Nog* gene expression (pro-neurogenic) in nasal mesenchyme [78]. Thus, BMP4 expression, by inducing *Nog*, actually defined the neurogenic permissive milieu in the

olfactory placode. In mice carrying reduced *fgf8* expression, expansion of BMP4 altered *noggin* expression away from the region where GnRH-1 cells normally arise (respiratory/olfactory epithelium border). Though through which mechanism *Fgf8* alters BMP expression is still unclear. Olfactory sensory cells still developed but GnRH-1 cells were absent. These experiments indicate that perturbing one signal during development cascaded forward to lead to a loss of a specific cell type, GnRH-1 neurons. Thus, correct formation of craniofacial structures (cartilage, mesenchyme and bones) is necessary for defining the neurogenic niches in the developing olfactory pit [55; 99; 104; 105]. These results imply that a broad analysis of mutations affecting craniofacial morphogenesis could reveal new important aspects at the base of olfactory sensory loss and HH.

The migratory track

The exact nature of the scaffold upon which GnRH neurons migrate on from the nose to the brain is still poorly defined. Olfactory sensory axons, vomeronasal sensory axons, terminal nerve axons, neuronal cells, glial cells, transient axons, and blood vessels have all been identified to bundle together [5]. The terminal nerve is defined as a cranial nerve that serves as part of the accessory olfactory system with a role in modulating the activity of the olfactory epithelium [109]. At early developmental stages, specific markers (with consistent expression throughout species) have not yet been found for most of the axonal groups crossing from the olfactory placode to the brain. Moreover, a number of different cell types have been described migrating at the same time and along a similar route to the one of GnRH-1 neurons ([51]). The various markers expressed by these migrating cells include olfactory marker protein (OMP) [110], gamma-aminobutyric acid (GABA) [111], tyrosine hydroxylase (TH), acetylcholinesterase NOS, neuropeptide Y (NPY) [109; 114], cardioexcitatory tetrapeptide (FMRF-amide), galanin neuropilin-1 and neuropilin-2 [117; 118; 119]. The fate of these migratory cells still needs to be clarified, as well as their physiological function, if any. A number of molecules such as stromal cell-derived factor 1 (SDF1)/chemokine receptor (CXCR-4), GABA, Hepatocyte growth factor (HGF)/Met receptor, PlexinB1, Semaphorins (3A, F, 4D, 7A), Nasal embryonic LHRH factor (NELF) and CCK, have been shown to influence the migration of GnRH-1 neurons [51; 117; 118; 119; 120; 121]. Several recent reviews of molecules involved in the migration of GnRH cells are available [51; 122; 123; 124]. However the cellular/molecular mechanism through which specific genetic mutations influence GnRH neuronal migration is often hard to unravel, due to the complex anatomy of the nasal region and broad expression of the same molecules among olfactory axons, migratory cells, mesenchyme and endothelial cells of blood vessels.

Kallmann syndrome

Many developmental aspects of the GnRH system that control the reproductive axis still need to be addressed e.g. embryonic lineage of the initial progenitor cells as well as whether different GnRH lineage derived-subpopulations play distinct physiological roles. However, it is broadly accepted in mammals, at least after specification, that 1) GnRH-1 neurons migrate from the nasal area to the central nervous system, 2) GnRH-1 neuronal migration is axophilic in that they use axons of the terminal nerve/olfactory pathways to reach their final

position [120; 125], and 3) GnRH-1 neurons migrate with other migratory neurons [126] and olfactory ensheathing cells [127; 128]. KS patients have mutations that alter 1 or more of these events.

It is known that KS is a developmental pathology with complex and heterogeneous genetic etiology [129; 130]. Observations, using different mouse models, indicate that syndromic anosmia and defective GnRH neuronal development may result from very different developmental events. Mouse models (summarized in Fig. 6), suggest that KS phenotypes can occur from gene mutations that broadly affect placodal development and therefore correct neurogenesis in the nasal area and craniofacial development. These kinds of mutations will impair the onset of GnRH neurons as well the onset of olfactory vomeronasal/terminal nerve cells [75; 76; 131; 132]. Genes in this category include *Fgf8* (see section above) [76; 101; 102; 103; 133], fibroblast growth factor receptor 1 (*FGFR1*) [134; 135; 136], 'Fgf8 synexpression genes' [137], *Pax6* [138; 139], and chromodomain helicase DNA-binding protein 7 gene (*CHD7*) [131; 132; 140; 141].

KS has also been linked to genetic mutations affecting cell adhesion molecules, molecules involved in neuronal path finding, and cell migration in nasal areas (mouse models in Fig. 6C). These genes include anosmin-1 (*KAL-1*) [129; 142], nasal embryonic LHRH factor NELF [142; 143; 144], SEMA7A [146; 147; 148], chromodomain helicase DNA-binding protein 7 gene (*CHD7*) [131; 132; 140], SEMA3A [149] and SOX10 [150]. *Kal-1* was the first gene identified to co-segregate with the KS phenotype but its mechanism of action in mammals is still unclear and difficult to investigate, as *Kal-1* is absent from the mouse genome [151]. *Kal-1* gene encodes for a secreted heparin-binding protein (anosmin-1) that interacts with multiple heparan sulfate proteoglycans and has a role in neuroblast migration [152; 153]. Inactivation of *Kal-1* in zebrafish and medaka was observed to affect fasciculation and targeting of olfactory sensory neurons, and to disrupt forebrain GnRH neuronal migration [42; 64; 89]. Notably recent data in chicken showed a key role for Anosmin in modulating FGF8 and BMP5 gene expression, cranial neural crest formation, and craniofacial development.

As suggested by mouse models in which prokineticin 2 ligand and receptor (*PROK2/PROKR2*) [155; 156; 157; 158; 159; 160], SEMA3A [149] or SOX10 genes have been knocked out or mutated [150], other forms of KS may be due to failure of the olfactory/terminal nerve fibers to establish proper contact with the forebrain [117; 147; 158; 161]. Lack of these fibers connecting with the brain can result from cell autonomous defects in olfactory neurons or defective formation of olfactory bulb (OB). Impaired OB development is a common phenotype in patients with KS. For a long time, OB induction was thought to depend on connections of olfactory fibers to the brain and not that OB induction influenced olfactory fiber connections [162; 163; 164; 165]. However, it is now known that in mouse lines where the olfactory epithelium fails to form or cannot innervate the brain (e.g. *Pax-6* *Sey^{Neu}/Sey^{Neu}* and *Dlx5* KO), OB-like structures still develop [166; 167] while in *PROKR2/PROK2* mouse mutants the OBs do not form correctly and the olfactory fibers fail to connect to the brain [158; 168]. These mouse models suggest that genetic mutations affecting central components of the olfactory system could be the cause for both anosmia

and HH in specific forms of KS [158; 168] such as mutations in *PROKR2*, a gene that is involved in both monogenic recessive and digenic/oligogenic KS transmission modes [157].

Olfactory ensheathing cells are they new players in KS defects?

OECs are the glial population of the olfactory system. Recently, two groups [169] [80; 83] showed that OECs, like Schwann cells of the peripheral nervous system, are neural crest derivatives (Fig. 7A, B). Notably, OECs being neural crest derivatives challenged the dogma that the olfactory system was composed of only placodal derivatives and has offered new insight into interpreting human pathologies such as KS that often include multiple neural crest defects.

The transcription factor *SOX10*, a member of the SRY-related HMG-box family, has a key role in defining the identity of several neural crest-derived cell populations, including Schwann cells and OECs [170; 171; 172; 173]. Recent studies showed that *SOX10* is required for terminal glial differentiation [170; 172; 174] and that loss of function of the *Sox10* gene leads to complete absence of mature Schwann cells and impaired OEC differentiation [171; 175]. Animal models [176; 177; 178; 179] have linked *SOX10* gene mutations to impaired maturation of OECs, tangle formation and misrouting of some olfactory axons, and defective GnRH-1 neuron migration to the brain (Figure 7C,D). Genetic studies in humans have linked *SOX10* gene mutations to demyelinating neuropathies and Waardenburg-Hirschsprung syndrome [176; 177; 178; 179]. This syndrome is a rare genetic disorder characterized by a spectrum of developmental defects that include pigmentation abnormalities in hair, eyes and skin, craniofacial defects, deafness, various neurologic manifestations and olfactory bulb agenesis. Examination of KS patients with deafness revealed that ~1/3 of these patients carry a *SOX10* loss-of-function mutation, indicating a substantial involvement of this gene in some forms of KS [150].

Although OECs share a high level of genetic and molecular similarities with Schwann cells [127; 181; 182; 183; 184; 185; 186; 187; 188; 189; 190], OECs do not myelinate olfactory axons but surround (ensheath) them as they exit the olfactory epithelium and project to the olfactory bulb [162; 163; 190; 191]. OECs are found in close apposition to developing olfactory axons from the earliest stages of olfactory development, E10.5-E11.5 in mouse [81; 192]. At this stage, pioneer olfactory axons, terminal nerve fibers and migratory neurons (together forming the migratory mass) start to emerge from the olfactory epithelium [192; 193], also reviewed in [81]. GnRH-1 neurons, as part of the migratory mass, travel together with other neuronal cells from the olfactory pit apposed to growing fibers and OECs [127; 192] (Fig. 7E). A potential guidance role for maturing OECs or OECs precursors in the migratory mass was suggested almost two decades ago [194]. OECs play an active role in growth, fasciculation, axon motility and patterning of primary olfactory neurons [191; 195; 196; 197; 198; 199; 200]. Since OECs extend processes early in development, it is possible that olfactory axon growth and pathfinding, is influenced by OECs. Notably some of the molecules known to be crucial in controlling GnRH-1 neuronal migration, such as Semaphorin 4D, NELF/Jacob, and SDF-1 α [43; 142; 143; 144; 145; 201] are expressed by OECs [127; 202; 203] (Fig. 7F). Thus, OECs might be important sources of factors needed for appropriate migratory mass movement [127; 204]. However, it is worth

noting that even in *Sox 10* gene null mouse embryos, ~20-30% of the total GnRH-1 neuronal population migrated into the brain, and OECs precursors (even if in an immature state) were identified along the olfactory axon/GnRH-1 neuron migratory route to the brain. Thus, as one would predict for such an important physiological system, multiple and probably redundant mechanisms are in place to ensure enough GnRH-1 neurons [205] enter the brain for reproductive competency.

From the examples above, it is clear that the neural crest derived OECs may be important players in both normal and abnormal olfactory development and GnRH-1 neuronal migration. As such, the role of these cells in the etiology of KS defects needs to be further investigated. In fact, HH can be syndromic with a number of neurological symptoms such as bimanual synkinesia (mirror movements of the hands), abnormal visual spatial attention, ocular motor abnormalities, sensory neural hearing loss and various degrees of cerebellar ataxia. These “satellite” defects could be, as *Sox10* gene mutations suggest [150; 171; 177], manifestations of broad gliopathies affecting both central and peripheral system, and therefore a more global cell type to consider in HH.

Conclusions

In the last thirty years we have reached a better understanding of the cellular composition of the developing olfactory area, development of the GnRH-1 cells and perturbations leading to HH/KS. However, we still do not fully understand the cell types involved in GnRH-1 neuronal development. Analysis of different animal models have revealed that GnRH-1 and its paralogs are broadly and differentially expressed in the animal kingdom. Evidence indicates that GnRH peptide isoforms that control gonadotropin function are associated with the olfactory/terminal nerve system. A mix embryonic lineage or genetic heterogeneity has been shown for GnRH-1 neurons and for the cells of the olfactory/vomeroneasal systems [80; 82; 83; 84]. The physiological functional significance of this, if any, is currently unknown. New evidence suggests that the OECs and certainly the nasal mesenchyme are important for normal GnRH neuronal development. These newly discovered developmental relationships highlight unexplored genetic mutations that may underlie the etiology and associated symptoms of HH and as such, may allow better clinical diagnosis and potential classification of the related syndromes. As we identify new molecules that are important for craniofacial development and olfactory/GnRH neurogenesis, such as *Fgf8*, we need to understand if KS and normosmic forms of HH reflect a spectrum of neurocristopathies as suggested by “satellite” defects such as lack of teeth, plate cleft, craniofacial defects, pigmentation defects and other neurological defects. Clearly, early changes in neural crest development and craniofacial development can lead to acute disruption in factors balancing GnRH neurogenesis and, as such, be the direct cause of some forms of HH.

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ABBREVIATIONS USED

GnRH-1	Gonadotropin Releasing Hormone
HPG	Hypothalamic–Pituitary–Gonadal
LH	luteinizing hormone
FSH	and follicle stimulating hormone
GnRHR	Gonadotropin Releasing Hormone Receptor
HH	hypogonadotropic hypogonadism
TN	terminal nerve
OMP	olfactory marker protein
KS	Kallmann syndrome
OB	olfactory Bulb
OECs	Olfactory ensheathing cells
OP	
E	embryonic day
FGF8	fibroblast growth factor 8
BMP	bone morphogenic protein
BLBP	Brain lipid-binding protein

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Highlights

- A general overview of the Hypothalamic–Pituitary–Gonadal (HPG) axis is provided
- What are the different forms of GnRH peptides in vertebrates?
- Developmental defects linking anosmia and hypogonadotropic hypogonadism in mouse models and humans
- Neural crest derivatives such as craniofacial tissues and olfactory ensheathing cells play key roles in controlling neurogenesis and migration of the GnRH neurons.

Fig. 1. Hypothalamic-pituitary-gonadal (HPG) axis

GnRH-1 cells extend their axons to the median eminence where they release GnRH-1 into the portal capillary system. Gonadotrope cells of the anterior pituitary, synthesize and release gonadotropins, LH and FSH. Pituitary release of FSH and LH, control sex steroid synthesis in the gonads.

Fig.2. Schematic illustrating the different GnRH paralogs expressed by vertebrate species. * indicates hypophysiotropic form.

Fig. 3. GnRH neuronal migration in the developing nasal region in mouse

Left panels: schematics of E11.5 and E12.5 mouse sections showing developmental changes in the nasal placode (red). Nose is toward the left. Right panels – corresponding stage immunocytochemically stained for GnRH (blue, arrowheads) and peripherin (sensory axon marker, brown). GnRH neurons are apposed to axons as they migrate from the VNO to the brain. OE=olfactory epithelium, RE = respiratory epithelium, VNO= vomeronasal organ, III= 3rd ventricle, FB = forebrain. NA=nasal area.

Fig.4. Cartoons summarizing key developmental studies (see references in figure) indicating the origin of GnRH neurons from the olfactory pit (A, B) and embryonic (C,D) and genetic (E, F) lineage of the progenitors

Though studies have been performed on different animal models the shape of the olfactory pit was adapted to the one of the mouse.

Fig.5. Fgf8 affects craniofacial growth and craniofacial signals

A) (E11.5) Whole head of Fgf8LacZ knock-in mouse showing Fgf8 (blue) is expressed in the rostral portion of olfactory pits (OP); MP-maxillary process. M=Medial; L= Lateral. B,C Coronal and parasagittal sections of E11.5 mouse nasal sections reveal that Fgf8 expression is limited to the non neurogenic portions of the olfactory pit, putative respiratory epithelium (RE), negative for the neuronal marker HuC/D (brown). R=rostral; D=dorsal; C=caudal; V=ventral. D-F) BMP4, NOG and neurogenesis. BMP4 (gray) represses neuronal cell fate acquisition leading surface ectodermal cells to acquire epidermal fate. BMP4 directly induces Nog expression (pink). Nog, by blocking BMP4, defines neurogenic permissive areas (green). Schematic, frontal view, mouse nasal area at E10.5 (based on [78; 99]) in control (E) and Fgf8 hypomorph (F). Mesenchymal BMP4 (gray) induces Nog expression (pink) in the medial and lateral mesenchyme. These sources of Nog define neurogenic areas (green) of the olfactory pit (OP). The GnRH neurons niche (light green) is defined by ventral mesenchymal sources of Nog. In response to reduced Fgf8 gene expression (F), facial BMP4 expression expanded, causing changes in Nog gene expression pattern and the neurogenic permissive areas of the olfactory pit (compare green areas and dotted line in E and F). The GnRH neurogenic niche is lost in Fgf8 mutants as Nog moves from the ventral position of the pit.

Fig. 6. What happens in KS?

Schematic of mouse embryo: (A) normal projections of olfactory, vomeronasal/terminal fibers (light blue) from the olfactory epithelium (OE) and VNO to the olfactory bulb (OB); GnRH neurons (green) migrating along the putative terminal nerve (TN) to the preoptic area of the hypothalamus (POA). B,C Two different KS developmental scenarios. B) Mouse models with defective olfactory pit formation or olfactory, vomeronasal and/or GnRH-1 neuronal onset. C) Mouse models carrying mutations that affect olfactory/terminal nerve growth or path finding. Defects in OB formation can be secondary to defective neuronal projection to the brain or directly affect the ability of the olfactory fibers to connect to the brain. These cartoons summarize mouse models that recapitulate KS phenotypes linked to mutations on the listed genes.

Fig. 7. The olfactory system is composed of placodal and neural crest cells. The OECs are important players in GnRH migration

A) Section of mouse embryo E11.5 immunostained against HuCD (green, neurons) and Sox10 (Red, olfactory ensheathing cells (OECs)). Over development, the migratory mass (MM) composed of neurons and glia moves away from the olfactory (OE)/vomeronasal epithelia (VNO) toward the brain. B) Crect/RosaYFP genetic lineage tracing reveals placodal derivatives as positive for YFP expression (green). The OECs of the migratory mass are not derived from placodal progenitors. C and D) Diagrams (based on [150; 175]) representing normal GnRH neurons migrating along the olfactory/terminal nerve fibers from the nose to the brain in control animals; (C) In Sox10^{null} mice, where the OECs do not complete maturation (pink dots), fewer GnRH neurons migrate into the brain and olfactory sensory axons misroute/defasciculate (D). Olfactory, vomeronasal and terminal fibers (blue), GnRH neurons green, OECs red in control, pink OECs precursors in Sox10^{null} mutant. E) BLBP is expressed by the OECS; X-Gal staining (blue) on a section from BLBPCreIRESLacZ, [207], embryo, E12.5 immunostained for GnRH-1 (brown). GnRH-1

neurons migrate (brown) in close contact to maturing OECs (blue nuclei) (see insert). F)
Model based on [127] representing OECS releasing molecules important for GnRH neuronal
migration/motility on olfactory/terminal fibers.

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