Clinical Manifestations of Impaired GnRH Neuron Development and Function

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Abstract

Gonadotropin-releasing hormone (GnRH) and olfactory neurons migrate together in embryologic development, and disruption of this process causes idiopathic hypogonadotropic hypogonadism (IHH) with anosmia (Kallmann syndrome (KS)). Patients with IHH/KS generally manifest irreversible pubertal delay and subsequent infertility due to deficient pituitary gonadotropins or GnRH. The molecular basis of IHH/KS includes genes that: (1) regulate GnRH and olfactory neuron migration; (2) control the synthesis or secretion of GnRH; (3) disrupt GnRH action upon pituitary gonadotropes, or (4) interfere with pituitary gonadotropin synthesis or secretion. KS patients may also have midline facial defects indicating the diverse developmental functions of genes involved. Most causative genes cause either normosmic IHH or KS except FGFR1, which may cause either phenotype. Recently, several balanced chromosomal translocations have been identified in IHH/KS patients, which could lead to the identification of new disease-producing genes. Although there are two cases reported who have digenic disease, this awaits confirmation

in future larger studies. The challenge will be to determine the importance of these genes in the 10–15% of couples with normal puberty who have infertility.

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Introduction

Reproductive function in humans depends upon the coordinated interaction of the hypothalamic-pituitary-gonadal axis directed by gonadotropin-releasing hormone (GnRH). GnRH is a decapeptide that is released from GnRH neurons in a pulsatile fashion into the hypophyseal-portal circulation, the capillaries of which perfuse the anterior pituitary gland. GnRH binds to the GnRH receptor, a G-protein coupled receptor on the cell surface of the gonadotropes, which then in turn synthesize and secrete the gonadotropins luteinizing hormone (LH) and follicle-stimulating hormone (FSH). The pituitary gonadotropins are released into the circulation, where they stimulate gonadal steroidogenesis and gametogenesis (fig. 1). The production of gonadal steroids, principally testosterone in males and estradiol in females, has negative feedback effects upon the hypothalamus and pituitary. A variety of peptide hormones including inhibin A and B, follistatin, as well as growth factors are also involved in this complex endocrine axis.

Fig. 1. Embryologic development of the GnRH pulse generator and the hypothalamic-pituitary-gonadal axis. Olfactory and GnRH neurons travel from the olfactory placode region to cross the cribriform plate. Some olfactory neurons go dorsally to the olfactory bulb, while others with GnRH neurons go ventrally to the forebrain which will become the hypothalamus. The location of migratory GnRH neurons (NLT and GN11) are indicated by *** while the postmigratory hypothalamic GT1-7 cells are indicated by ##. The negative sign (-) indicates negative feedback of sex steroids on the hypothalamic-pituitary axis.

Defects in the development of the hypothalamus, impairment of GnRH and olfactory neuron migration, and deficient GnRH secretion and/or action not only would be predicted to have detrimental effects upon pubertal development and fertility, but have been demonstrated to do so in humans. Mutations in genes affecting GnRH neuroendocrine function demonstrate the importance of these particular genes in normal human reproductive function.

Factors Governing GnRH and Olfactory Neuron Migration

In teleost fish and mammalian embryological development, GnRH neurons migrate with olfactory neurons from the olfactory placode region to their normal position in the hypothalamus [1]. The vomeronasal nerve originates outside the brain and travels from the olfac-

tory epithelium to cross the cribriform plate and then sends fibers dorsally to the olfactory bulb (fig. 1). The caudal vomeronasal nerve traverses more ventrally with GnRH neurons into the forebrain region that becomes the hypothalamus. The specific details of the precise regulation of GnRH and olfactory neuron migration are unknown, but experimental evidence supports the involvement of a number of factors. The netrin signaling system has been implicated in GnRH neuron migration since both of the knockout mouse models for the ligand netrin-1 and one of its receptors – deleted in colorectal carcinoma (DCC) – demonstrate impaired GnRH neuron migration [2, 3]. In addition, both netrin-1 and DCC are expressed in GnRH neuronal cell lines. Other factors known to affect GnRH neuron migration include: hepatocyte growth factor/scatter factor (HGF/SF), Gas6/Ark signaling, glycoconjugates, slit, gamma aminobutyric acid (GABA), as well as neural cell adhesion molecules and related proteins (tenascin, phosphacan, and laminin) [1].

Four factors appear to have clinical significance in humans, and these will be discussed in more detail below. There is evidence from both in vitro analyses and translational findings in human disease that both anosmin-1, encoded by the KAL1 gene, and fibroblast growth factor (FGF) signaling affect both olfactory and GnRH neuron migration. Mutations in these genes cause Kallmann syndrome, the combination of idiopathic hypogonadotropic hypogonadism (IHH) and anosmia (see below). There is also presumptive evidence that nasal embryonic LHRH factor (NELF) and chromodomain helicase DNA-binding protein 7 (CHD7) may cause Kallmann syndrome, but this is not as conclusively established as it is for KAL1 and FGFR1. The important embryologic event of GnRH and olfactory neuron migration illustrates the close interaction of the reproductive and olfactory systems, which when disturbed causes reproductive and olfactory dysfunction.

In vitro Analysis of GnRH Neuron Migration

Studying the functional effects of mutations upon GnRH neuron migration and function has been challenging because of the small number of GnRH neurons (~800 in mice and 1,000–2,000 in primates), which are dispersed throughout the brain. The generation of two types of immortalized GnRH neurons has permitted the study of more immature, migratory neurons, as well as the mature, postmigratory GnRH neurons. To generate

hypothalamic specific tumors, Mellon et al. [4] inserted the rat *Gnrh1* promoter upstream of the SV40 T antigen and injected this construct into fertilized mouse eggs to produce transgenic mice. Hypothalamic tumors were identified, and the resulting nonmigratory GT1-7 cells were found to express and secrete pulsatile GnRH. Similarly, Radovick et al. [5] cloned the human *GNRH1* promoter upstream of the T antigen. This time, the transgenic mice demonstrated tumors along the olfactory placode/olfactory region that did not cross the cribriform plate. These cells (NLT and GN11) are migratory, but secrete much less GnRH than the postmigratory GT1-7 cells (fig. 1). Although cell lines cannot fully recapitulate in vivo conditions, they provide a reasonable starting point to try to understand the mechanisms of GnRH action.

The Ontogeny of GnRH Secretion

Although GnRH neurons are dispersed throughout the central nervous system, the arcuate nucleus contains the GnRH neurons that appear to regulate puberty and reproduction. Since GnRH is secreted in a pulsatile fashion (with a very short half-life of 1–3 min) within the hypophyseal-portal system and directs the reproductive axis, this is often referred to as the ‘GnRH pulse generator’. The activity of the GnRH pulse generator is reflected by changing gonadotropin levels during the human lifespan. In the human embryo, gonadotropins begin to rise at 10 weeks of gestation, peak in the second half of pregnancy, but then decline because of the negative feedback from placental steroids. After birth when gestational steroids are removed, both FSH and LH rise in the neonatal period and remain elevated for different times, which are sex-specific (the first 6 months in boys and the first 1–2 years in girls), with a subsequent decline during childhood until puberty ensues [6]. This decline in gonadotropin secretion appears to be central in origin since patients with gonadal failure have low/normal levels of gonadotropins during childhood prior to the time of puberty. Molecular mechanisms that control the initiation of puberty are incompletely understood, but evidence from human mutations suggests at least two different pathways – leptin and kisspeptin/GPR54 signaling are important in normal puberty.

About age 9 years, gonadotropins begin to rise, first FSH, then LH, which reflects the frequency of the GnRH pulse generator. Slower GnRH pulses appear to preferentially favor FSH secretion, while more rapid pulses favor

LH secretion. In early puberty, LH pulses are nocturnal, but subsequently occur throughout the 24-hour period. This increased pulsatile secretion of LH (along with FSH) stimulates gonadal sex steroid secretion, which then produces negative feedback upon the hypothalamus and pituitary gland [6]. In adult males, LH pulses occur roughly every 2 h, but vary in adult females depending upon the time of the menstrual cycle. Typically pulses occur every ~90 min in the early follicular phase, but increase to hourly pulses near the LH surge. Following ovulation in the luteal phase, LH pulses slow down to every 2–4 h.

Normal Puberty

Normal puberty is usually a progression of events, which when initiated, is typically completed in 3–4 years [7]. Adrenarche, the secretion of adrenal androgens (androstenedione, DHEA, and DHEAS), begins about 6–8 years of age and continues through the mid-teens in both sexes. In girls, breast development (thelarche) generally begins about age 8–9 years, about the same time of pubic hair development (pubarche). A growth spurt occurs at 12 years of age, which on the descending arm of the growth spurt the first menstrual cycle (menarche) occurs [7, 8]. In boys the initial pubertal event is testicular growth, which usually begins about 10.5 years of age when the testes are at least 2.5 cm in any dimension. Pubarche frequently starts simultaneously with testicular development, while axillary hair growth occurs at about the time of peak height velocity (age 14 years).

Delayed Puberty

Overview of the Evaluation

Delayed puberty is usually defined as the absence of signs of puberty by age 14 in boys and by the absence of breast development by age 13 or menarche by age 15 in girls. The etiology of delayed puberty is complex, and will not be detailed here. However, thyroid disease and hyperprolactinemia should be excluded in all patients, regardless of whether they are hypogonadal or not. In hypogonadal patients (males or females with low sex steroids), serum gonadotropins help classify the site of the HPG axis that is disrupted [7, 8]. If LH and FSH are elevated on several occasions, the diagnosis is hypogonadotropic hypogonadism (or gonadal failure) and a karyotype is necessary to exclude chromosomal abnormalities (47,XXY or 46,XX in males; 45,X or a 46,XY cell line in females).

Hypogonadotropic Hypogonadism

Alternatively, if the patient with hypogonadism has low or normal FSH and LH, hypogonadotropic hypogonadism is present, signifying hypothalamic or pituitary dysfunction [7, 8]. In these patients, a pituitary tumor, typically a prolactin-producing adenoma or a craniopharyngioma, must be excluded by MRI. Extreme weight loss/eating disorders, stress, and excessive exercise must be excluded, as shown by a number of chronic medical conditions (malignancy, renal disease, neurologic diseases, etc.). If these conditions are not present, the more reversible form of constitutional delay of puberty (CDP) must be differentiated from the more severe phenotype of isolated or idiopathic hypogonadotropic hypogonadism (IHH). If hypogonadotropic hypogonadism is present ≥ 17 years for girls and ≥ 18 years for boys, the diagnosis is IHH [9]. If there is impairment in olfaction (anosmia), then Kallmann syndrome (KS) is present. Combined pituitary hormone deficiency (CPHD) should be considered if extreme short stature is also present, since it could indicate severe pituitary failure with growth hormone and thyroid hormone deficiency.

Clinical Features of Idiopathic Hypogonadotropic Hypogonadism and Kallmann Syndrome

Although somewhat arbitrary and not always easy to determine clinically, IHH may be classified according to the severity of sex hormone deficiency. Without any evidence of sexual development, the patient may be classified as complete IHH, and if there is some evidence of puberty, incomplete IHH [10]. This distinction must be determined prior to the patient ever receiving hormone therapy. Females with no breast development (Tanner stage I) and males with testes $\leq 3 \text{ cm}^3$ have complete IHH, while those females with some evidence of breast development and males with testes $>3 \text{ cm}^3$ (15–25 cm^3 normal) have incomplete IHH. This sub-classification has been reported to be more valuable than endocrinological studies since gonadotropins and serum sex steroid levels do not differentiate between the complete and incomplete forms of IHH [10].

A variety of somatic anomalies may also be associated with hypogonadotropic hypogonadism such as anosmia/hyposmia, cleft palate, dental agenesis, visual abnormalities, deafness, mental retardation, renal agenesis, pes cavus, and neurologic abnormalities such as cerebellar dysfunction and synkinesia. Since some patients with CHARGE syndrome (coloboma of the eye, heart defects,

choanal atresia, retardation in growth and development, genitourinary abnormalities, ear anomalies) appear to have anosmia and endocrine studies suggestive of IHH/KS, these anomalies must also be considered (see below). IHH/KS may be associated with a variety of chromosomal abnormalities and other Mendelian disorders, thereby complicating the understanding of this diagnostic entity.

Treatment of IHH/KS involves the use of either estrogen for breast development in women or testosterone in men for secondary sex characteristics. When fertility is desired, pulsatile GnRH may be administered, but in practice, it is more common to use gonadotropins (both FSH and LH) for ovulation induction in women and for spermatogenesis in men. Generally, women ovulate and their cycle fecundity is normal for their age. For men, treatment usually takes 6–12 months, but improvements in sperm production are evident in most men [11]. Although IHH/KS has always been thought to be irreversible and lifelong steroid hormone treatment was advocated (except when considering pregnancy), it has recently been reported that perhaps as many as 10% of men with IHH or KS may resume LH pulsatility and normalize their testosterone levels when treatment is discontinued [12].

Molecular Basis of IHH

Pedigree Structure

The prevalence of IHH/KS in the general population is unknown, but is estimated to be 1/10,000 males and 1/40,000 females. Likewise the proportion of familial cases is unclear and probably affected by the method of ascertainment. Waldstreicher et al. [13] found that 18% of 106 unrelated IHH probands had ≥ 1 affected family members with IHH, but this was increased to 34% if reversible delayed puberty or isolated anosmia in family members were included. Quinton et al. [14] studied 215 IHH patients from three different London hospitals, and found familial transmission in 24% patients. However, Bhagavath et al. [15] found that only 6.3% of 315 unrelated IHH patients had a family member affected with IHH/KS. In all three of these studies, inheritance patterns included X-linked recessive, autosomal recessive, autosomal dominant [13–15].

Chromosome Rearrangements

Normal management of patients with IHH/KS does not usually include a karyotype unless there is suspicion for Prader-Willi syndrome (60–70% have a deletion

Table 1. Reported chromosomal rearrangements with IHH/KS

Chromosomal rearrangement	Phenotype	Author
t(7;12)(q22,q24)	KS	Best et al., 1991 [178]
X;Y	KS	Guioli et al., 1992 [179]
t(3;9)(9;12)(q13.2;q21.2p13;q15)	KS	Casamassima et al., 1993 [180]
t(13;16)(q14.11;q24)	IHH	Kikuchi et al., 1993 [181]
t(4;12)(q25;q24.2)	hypogonadism?	Elbistan et al., 1994 [182]
t(1;10)(q44;q26)	KS	Schinzel et al., 1995 [183]
46,XY,inv(3)(q24q26.32),t(3;13;18)(q26.32;q21.2;q12.2)	KS	Kroisel et al., 2000 [184]
del8p11.2	KS; spherocytosis	Vermeulen et al., 2002 [185]
46,XY,inv(10)(p15.2q11.22).ish inv(10)(p15.2q21.3)(p15 × 3), (q21 × 3)(p15conq21 × 2)(X)(p11.4q11.2)	IHH	Helszer et al., 2003 [186]
46,XY,t(7;8)(p12.3;p11.2)	IHH	Talaban et al., 2005 [187]
46,XY,t(10;12)(q26.3;q13.1)	IHH	Kim et al., 2005 [188]
46,XY,t(10;12)(q26.3;q13.1)	KS	Bhagavath et al., 2006 [15]
46,XY,mos t(3;12)(p13;p13)[18]/46,XY[3]	IHH and cerebellar ataxia	Bhagavath et al., 2006 [15]
46,XY/46,X,inv(Y)(p11.2q11.2)	IHH	Bhagavath et al., 2006 [15]

15q11-q13) or there are multiple congenital anomalies. Although some chromosomal translocations have been reported in IHH patients (table 1), no systematic study was performed until recently when 3/76 (4%) of IHH patients were found to have chromosomal abnormalities [15]. Of 76 IHH/KS patients studied, 73 were normal (54 46,XY and 19 46,XX). However, three males without a family history of IHH/KS had chromosome abnormalities: 46,XY,t(10;12)(q26.3;q13.1); 46,XY/46,X,inv(Y)(p11.2q11.2), and mos46,XY,t(3;12)(p13;p13)[18]/46,XY [3]. Balanced translocations may or may not cause the disorder [15], but provide valuable information for positional cloning of new IHH/KS genes.

Mendelian Forms of IHH

Mutations in the KAL1, GNRHR, and FGFR1 genes account for most of the known cases of IHH/KS so the genotype/phenotype correlations will be discussed in more detail. However, all known molecular causes of IHH/KS published in peer-reviewed journals will also be reviewed (table 2). To date, KAL1 gene mutations have been exclusively identified in anosmic males with KS, and GNRHR mutations have only been identified in normosmic IHH patients [16]. Although the molecular pathways of KS and normosmic IHH were initially thought to be independent, this has now been brought into question since fibroblast receptor-1 (FGFR1) mutations appear to cause both KS and IHH [17]. Two additional genes, nasal embryonic LHRH factor (NELF) and chromodomain helicase DNA-binding protein 7 (CHD7) appear to be prom-

ising candidates for mutations in KS, which, if true, would increase the number of known genes causing KS to be four – KAL1, FGFR1, NELF, and CHD7.

KAL1

Men and women with KS demonstrate IHH and anosmia, further supporting the common developmental pathway of GnRH and olfactory neurons. Mutations in the KAL1 gene on chromosome Xp22.3 were first described in males with X-linked recessive KS [18, 19]. The KAL1 gene spans ~203 kb of genomic sequence, contains 14 exons, and has a homologous pseudogene on the Y chromosome. KAL1 encodes anosmin-1, a putative neural cell adhesion molecule which appears to be involved in the migration of GnRH and olfactory neurons during embryologic development. Deletions were first identified in males with multiple congenital anomalies including KS with a contiguous gene deletion syndrome including the KS locus [18, 19]. Subsequently, intragenic deletions [20] and point mutations [21] were identified in KS patients, confirming that KAL1 mutations were causative.

The KAL1 gene has been cloned in a variety of species including *Drosophila*, *Caenorhabditis elegans*, zebrafish, and chicken, but surprisingly has not yet been identified in mice, despite the fact that the human antibody detects its presence [22]. Expression of KAL1 in both human and chick embryos demonstrates an excellent correlation to the tissues affected in KS patients. KAL1 mRNA has been identified in the olfactory bulb, retina, oculomotor nucleus, cerebellum, corticospinal tract, facial mesenchyme,

Table 2. Gene mutations causing and suspected of causing IHH/KS in humans

Gene	Localization	Phenotype	Inheritance
<i>GnRH neuron migration</i>			
KAL1	Xp22.3	KS	X-linked recessive
FGFR1	8p11.2-p11.1	KS or IHH	autosomal dominant
<i>Hypothalamic function</i>			
GPR54	19p13.3	IHH	autosomal recessive
LEP	7q31.3	obesity, IHH	autosomal recessive
LEPR	1p31	obesity, IHH	autosomal recessive
NROB1*	Xp21	adrenal hypoplasia congenita and IHH	X-linked recessive
PCSK1	5q15-q23	obesity, IHH	autosomal recessive
<i>Pituitary function</i>			
GNRHR	4q21.2	IHH	autosomal recessive
FSHB	11p13	isolated FSH deficiency	autosomal recessive
LHB	19q13.3	isolated LH deficiency	autosomal recessive
HESX1	3p21.1-21.2	septo-optic dysplasia	autosomal recessive
PROP1	5q	short stature, hypothyroid, CPHD	autosomal recessive
LHX3	9q34.3	CPHD	autosomal recessive
LHX4	1q25	CPHD	autosomal dominant
SOX2	3q26.3-q27	microphthalmia/midline CNS defects, CPHD	autosomal dominant, sporadic
SOX3	Xq26.3	midline CNS anomalies, CPHD	X-linked recessive
<i>Putative genes</i>			
NELF	9q34.3	IHH/KS	digenic/monogenic?
CHD7	8q12.2	CHARGE syndrome, KS	autosomal dominant, sporadic
PROK2	3p21.1	KS?	?
PROKR2	20p13	KS?	?

CPHD = Combined pituitary hormone deficiency. * NROB1 may have hypothalamic and pituitary effects.

meso/metanephros, and cartilage/limb buds [23]. Some patients with KS may also manifest a variety of nonreproductive abnormalities including renal agenesis and midline facial defects. In some patients, neurologic abnormalities may also be present, such as synkinesia (mirror movements of extremities), cerebellar dysfunction, deafness, eye abnormalities, and mental retardation. A careful search for midfacial and brain defects, along with documenting the presence of two kidneys by ultrasound, has become important in taking care of these patients. The expression patterns of KAL1 correlate closely with the associated anomalies identified in IHH/KS patients, thereby providing useful information to clinicians taking care of these patients.

Patients with KS appear to have a defect in migration of GnRH neurons, although the precise localization of the defect in olfactory and GnRH neuron co-migration is unknown. The function of anosmin-1 remains unknown, but it is a secreted protein containing a cysteine-rich region, a highly conserved four-disulfide core whey acid

protein (WAP) domain, four fibronectin type III (FNIII) repeats, and a C-terminal histidine-rich region [18, 19]. The WAP domain is common among protease inhibitors, which have been shown to play a role in axonogenesis and neuronal migration. FNIII domains are present in cellular adhesion molecules, extracellular matrix proteins, and protein tyrosine kinases and phosphatases that have been implicated in neuronal migration and in axon targeting [18, 19].

Experimental evidence supports these putative functions of anosmin-1. In the mouse, anosmin-1 stimulates axonal branch formation from olfactory bulb output neurons [24]. In *C. elegans*, the WAP domain influences axon targeting, and the FNIII region appears necessary for axon targeting and axonal branching [25]. Anosmin-1 has been hypothesized to provide a scaffold to direct migration of both olfactory and GnRH neurons to their proper anatomic locations. Disruption of wild-type anosmin-1 protein results in impaired neuronal migration and KS results. Documentation of impaired neuronal mi-

gration has been reported in the autopsy of one fetus with KS, further supporting this hypothesis [26]. Anosmin-1 has also been shown to directly stimulate GnRH neuron migration of mouse GnRH (GN11) neuronal cells in vitro [27], and knockdown of *kal1.1* (not the second gene *kal1.2*) in zebrafish interferes with GnRH neuron migration [28].

Mutations in *KAL1* account for 33–70% of familial cases of KS and 3.1–27.8% of apparently sporadic forms of IHH with anosmia [29]; [18–21, 30–46]. Up to half of males with *KAL1* mutations may have unilateral renal agenesis [46]. No *KAL1* mutations have been described to date in females although this has not been studied in a large sample. It is clear that some female IHH patients are anosmic or hyposmic, suggesting the involvement of other genes affecting olfactory development and GnRH function. As is true in human genetic disease, marked phenotypic variability may occur, even within families [33]. Some investigators have suggested that the phenotype of patients with *KAL1* mutations is more severe than in those with other causative mutations [34, 42]. Severity was defined by the presence of cryptorchidism, absent LH pulses, and lack of response to exogenous GnRH [34, 42]. Reported *KAL1* mutations described to date and their relation to the protein structure are reviewed elsewhere, and include deletions, missense, and frameshift mutations [18–21, 29–46]. Although three different missense mutations were tested to determine if they affect migration of GnRH neuronal cells (NLT cell), none reduced migration in vitro [27]. This in vitro assay does provide a possible method to test the functional significance of *KAL1* mutations.

FGFR1

The second gene for KS (*KAL2* locus) was found to be the *FGFR1* gene. The positional cloning of this gene was aided by the identification of two patients with contiguous gene deletion syndromes on chromosome 8p11.2–p12 who also had KS [47]. No additional gene deletions were identified in 43 KS patients, but 12/129 [9%] had heterozygous *FGFR1* point mutations [47]. Subsequent studies of both males and females with autosomal dominant IHH/KS have indicated that *FGFR1* mutations may cause either anosmic/hyposmic (KS) or normosmic IHH [17, 31, 32, 47–51; 52]. Current data suggest that *FGFR1* mutations occur in 10% of either anosmic or normosmic IHH. Some patients with *FGFR1* mutations may also have associated neurologic abnormalities and midfacial defects, similar to those with X-linked KS. As is true in many autosomal dominant diseases, a complicating and chal-

lenging feature among families with inactivating *FGFR1* mutations is reduced penetrance and variable expressivity [17, 31, 32, 47–51]. At least 39 mutations leading to IHH with or without anosmia have been described to date [17, 31, 32, 47–51]. More recently, functional analysis of mutations have demonstrated an impairment of FGF signaling in vitro [17, 51].

The human *FGFR1* gene is comprised of 18 exons contained within 56 kb of genomic DNA encoding a single transmembrane receptor consisting of three immunoglobulin-like (Ig) domains, a heparin binding domain, and two tyrosine kinase domains. *FGFR1* binds FGF1 and FGF2 with high affinity, involving a K18K sequence essential for receptor activity and interaction with heparin [53]. Since *FGFR1* and *KAL1* both utilize heparin sulfate proteoglycans, it is possible that anosmin-1 could be a ligand for *FGFR1*. The importance of the FGF pathway in GnRH neuron development has been demonstrated by a number of studies. *FGFR1*, along with *FGFR2* and *FGFR3*, is expressed in the nasal placode about E10.5 in the mouse, when GnRH neurons first appear. *FGFR1* and *FGFR3* are also expressed in GnRH neurons at E15.5 and postnatally in the mouse [54]. Additionally, an attenuation of neurite outgrowth has been described following stable transfection of an *Fgfr1* dominant negative mutant. These experimental findings indicate that FGF signaling is involved in GnRH neuron specification, migration, and axonal targeting [54].

It is interesting that human *FGFR1* mutations may be activating or inactivating. In fact, the first *FGFR1* mutations described were activating mutations that cause craniosynostosis and other skeletal anomalies such as craniofacial-skeletal dysplasia (Jackson-Weiss syndrome), Pfeiffer syndrome, trigonocephaly, osteoglophonic dysplasia, and Antley-Bixler syndrome [55–58]. However, inactivating mutations cause IHH/KS, but it is interesting that skeletal effects occur in both activating and inactivating mutations of the *FGFR1* gene.

GNRHR

Mutations in the GnRH receptor (*GNRHR*) gene were initially thought to be the most common cause of normosmic IHH [59–61], but now it appears that the frequency of *FGFR1* mutations might be greater [17, 51]. The *GNRHR* is a G-protein-coupled receptor (GPCR) localized to the cell surface of pituitary gonadotropes, which contains the characteristic seven transmembrane domains and intra- and extracellular loops, but notably lacks an intracellular cytoplasmic tail. When the GnRH ligand reaches the pituitary gonadotropes via the hy-

pophyseal portal system and binds to the GNRHR, second messenger inositol trisphosphate (IP_3) is released which then stimulates intracellular calcium release which in turn triggers gonadotropin synthesis and release.

The human GNRHR gene consists of three exons spanning 18.7 kb of genomic DNA, and was the first gene shown to have mutations in autosomal recessive IHH [59–61]. More than 21 different GNRHR mutations have been described in IHH, most of which are compound heterozygous missense mutations [34, 59–76]. Nearly all human GNRHR mutations occur in exons 1 or 3, but they are dispersed throughout different domains of the receptor (extracellular, transmembrane, and intracellular) [34, 59–76]. Two mutations comprise nearly half of the reported alleles, even among different ethnic groups – Gln206Arg mutation [32.6%] and Arg262Gln (15.2%) [59]. Just over half of the reported GNRHR mutations interfere with ligand binding, while the remainder affect signal transduction. In addition, some mutations impair gonadotropin subunit and GNRHR promoter activity. Interestingly, studies utilizing site-directed mutagenesis and in vitro analysis have shown that some GNRHR mutations appear to act as dominant negative mutations [77]. The clinical significance of this is unclear, since reported IHH patients have two mutant alleles while heterozygotes do not have any noticeable phenotype.

Findings from several large studies suggest that the prevalence of GNRHR mutations in all IHH patients ranges between 2 and 5% [59]. To date, there have been no reports of GNRHR mutations in anosmic or hyposmic IHH patients, indicating the prevalence of GNRHR mutations in normosmic IHH patients is between 3.5 and 10.6% [59]. In IHH families demonstrating clear autosomal-recessive inheritance, the prevalence is estimated to be between 6 and 11% [59].

In our recent review of GNRHR mutations, about 60% of the probands had complete IHH (no evidence of sexual development) [59]. The remainder had incomplete IHH, but the phenotype was unknown in several patients. Variable expressivity has also been reported even within the same family, since some affected patients may show some signs of pubertal development while others exhibit a total lack of pubertal development [59]. Endocrinologic findings may also vary for patients with the same mutations. Although successful ovulation and pregnancy with exogenous GnRH has been reported in patients with this mutation, no live births were achieved [65, 69]. It is likely that the status of other modifier genes, epistatic events and environmental factors influence the final phenotype of patients with GNRHR mutations.

Other Causes of IHH/KS

Hypothalamus

KISS1/GPR54

Although kisspeptins, which are ligands for the GPR54 (G-protein-coupled receptor 54), were originally isolated from melanomas (the first kisspeptin was termed metastatin), the significance of this pathway in reproduction did not become apparent until the positional cloning of the GPR54 gene in IHH patients. Independently, two groups studied consanguineous families by linkage analysis and demonstrated very high LOD scores for chromosome 19, where the GPR54 gene resides [78, 79]. In the Saudi Arabian family, DNA sequencing revealed a homozygous missense mutation, while in another unrelated patient, compound heterozygosity for a nonsense mutation (R331X) and a nonstop (X399R) was identified [79]. All three mutations demonstrated impaired signal transduction (diminished inositol phosphate production) in vitro [79]. A French group identified a homozygous missense mutation and a homozygous 155-bp deletion in two different normosmic IHH patients [78]. The missense mutation was later found to also impair GPR54 signaling [80]. No definitive mutations in the KISS1 gene have yet been identified in humans.

Patients with mutations that were studied had impaired GnRH secretion, but had some impaired gonadotropin response to pulsatile GnRH [78, 79]. Affected females respond well to gonadotropin for ovulation induction and achieve normal pregnancy, delivery, postpartum recovery and lactation [81]. As might be expected in an autosomal-recessive disease, heterozygous offspring appear to be normal, with the oldest known child, a 15-year-old girl, having normal puberty and menarche [81]. Further support for the role of GPR54 in normal puberty comes from the knockout mouse, which was hypogonadal. Homozygous knockout mice, however, had a normal anatomic location of GnRH neurons and normal GnRH content in the brain, suggesting that *GPR54* signaling is important in GnRH release [79]. A variety of additional rare sequence variants of GPR54, some of which are likely to be functional, have been identified, but their consequences are not completely understood [82–85].

Kisspeptins are most highly expressed in the placenta among other tissues, but they are also found in the anteroventral periventricular (AVPV) nucleus and the arcuate nucleus of the hypothalamus. Neurons from these nuclei send projections to the medial preoptic area of the hypothalamus where GnRH neurons reside and secrete GnRH into the hypophyseal portal system. It is known

that GPR54 is present in 50–75% of GnRH neurons [86–88]. The transition from the prepubertal state to puberty is marked by kisspeptin activation of GPR54 [89]. Kisspeptin infusions result in increased pituitary gonadotropin secretion, an effect that can be abolished by GnRH antagonist administration, indicating a hypothalamic effect of kisspeptin upon GnRH release [89–91]. Gonadectomy increases and sex steroid replacement decreases kisspeptin expression in the arcuate nucleus, whereas the opposite is true in the AVPV nucleus [92, 93]. These findings suggest that kisspeptin signaling may be important in steroid feedback, and that perhaps the LH surge may be influenced by differential effects of kisspeptin upon these hypothalamic nuclei.

LEP and LEPR

Leptin is a protein secreted by white adipose tissue that regulates body weight by decreasing food intake and increasing energy expenditure. Leptin is also involved in the modulation of neuroendocrine, reproductive, immune, and cardiovascular systems, particularly in the energy-deficient state. Pulsatile leptin secretion is maximal during the evening and early morning hours [94], and may exert its influence by acting upon KISS1 neurons in the arcuate nucleus [95]. Translational support for the role of leptin in regulating the HPG axis was reported by Welt et al. [96] who found that three of eight women with hypothalamic amenorrhea ovulated following a 3-month leptin infusion. Leptin binds to the leptin receptor (LEPR), a member of the cytokine family of receptors. Leptin signaling involves activation of the STAT3 pathway intracellularly and as well as interaction with the melanocortin 4 receptor (MC4R) to produce anorectic effects [97].

More definitive evidence of leptin's role in reproduction has come from the molecular analysis of individuals with severe, early-onset obesity. Mutations in leptin (LEP) [98–101] or in leptin receptor (LEPR) [102, 103] lead to hyperphagia and morbid obesity in early childhood. The first human LEP gene mutation was characterized in a family with two obese prepubertal children who had extremely low serum leptin levels, but all subjects were prepubertal [98]. Subsequently, a second LEP gene mutation was identified in a consanguineous family containing 3 affected obese individuals [99]. A 14-year-old female presented with primary amenorrhea and a 22-year-old male had irreversible pubertal delay, low testosterone, low gonadotropins, but a normal response to exogenous hCG and GnRH. Both affected family members were homozygous for a missense LEP gene mutation impairing leptin secretion in vitro [99]. The phenotype of humans with

LEP mutations is similar to leptin-deficient *ob/ob* mice since they manifest extreme obesity, hyperinsulinemia, and hypogonadotropic hypogonadism, but unlike the mice, the patients do not have hyperglycemia, hypercortisolemia, and stunted height [99]. Only 12 LEP mutations have been identified to date [104] in individuals with extreme, early-onset obesity, so the prevalence in all IHH/KS patients is likely to be very low. The presence of LEP mutations should be considered in IHH patients who are extremely obese and have low serum levels of leptin.

Leptin-resistant *db/db* mice have mutations of the *Lepr* gene, but until recently [104] only one LEPR mutation was described in humans [102]. Leptin resistance was observed in a family of severely obese females with hypogonadotropic hypogonadism and elevated serum levels of leptin. Homozygosity for a LEPR splice mutant resulted in exon-skipping, thereby truncating the receptor so that it lacked both the transmembrane and intracellular domains [102]. In addition to IHH, this patient also had mildly reduced secretion of GH and TSH [102]. Recently, a subset ($n = 300$) of a large cohort of 2,100 early-onset obese subjects with a BMI standard deviation score = 4.5 who were negative for mutations in known obesity genes were screened for LEPR mutations [104]. Eight of these 300 (2.7%) subjects had either missense or nonsense LEPR mutations, all of which impaired LEPR signaling in vitro. The affected patients demonstrated, in addition to severe obesity, hyperphagia, altered immune function, and delayed puberty secondary to IHH. Interestingly, serum leptin levels were not different from similarly obese patients without LEPR mutations, and somewhat surprisingly, the clinical features of patients with LEPR mutations were less severe than those with LEP mutations [104].

NROB1

Another X-linked cause of human IHH/KS is adrenal hypoplasia congenita (AHC)/IHH caused by mutations of the NROB1 gene [105]. This gene was cloned from the dosage sensitive sex reversal (DSS) region of chromosome Xp21 in humans, which when duplicated has been reported to cause undermasculinization in 46,XY males [105, 106]. The identification of NROB1 gene was aided by the findings from patients with a contiguous gene deletion sequence on Xp21 manifesting Duchenne muscular dystrophy and glycerol kinase deficiency in addition to AHC/IHH. The human NROB1 gene encodes for the DAX1 (DSS-AHC critical region of the X chromosome, gene 1) protein, which is a member of the steroid hormone receptor superfamily which has no known ligand

(an orphan receptor). DAX1 is a transcription factor necessary for normal development of the hypothalamus, pituitary gonadotropes, and adrenal cortex [105, 106].

Males with NROB1 mutations usually present with adrenal failure in infancy or childhood due to an underdeveloped permanent zone of the adrenal gland [105–112]. Children that are adequately treated with adrenal steroids have delayed puberty due to IHH. Occasionally, patients develop adrenal insufficiency later in life or only after targeted testing of the adrenal gland [110–112]. Unfortunately, patients with NROB1 mutations do not respond well to gonadotropins and may show minimal response to pulsatile GnRH, implicating an inherent defect in the gonads [113, 114]. A large variety of different NROB1 mutations, including deletions and point mutations, have been reported in AHC patients, which have been almost completely confined to males [105–112, 114–122]. Of interest though, a homozygous NROB1 mutation was reported in an IHH female without adrenal failure within a family containing two males with AHC. Her homozygosity was thought to be due to a gene conversion event. Female carriers with DAX1 mutations may manifest delayed puberty [109].

DAX1 appears to regulate gonadotropin secretion at both hypothalamic and pituitary levels [122, 123]. DAX1 may also inhibit transcription of another important steroid receptor, SF-1, which is expressed in the same tissues and is important in sexual differentiation [123]. Although initial studies suggested that NROB1 might be an ovarian determinant gene because of its localization within the DSS region on Xp, a conditional knockout of the mouse ortholog of NROB1 (*Nrob1*) did not confirm this [124]. These mice demonstrated normal ovarian development and function; however, male mice displayed degeneration of testicular germinal epithelium independent of gonadotropin and testosterone abnormalities [124]. These findings demonstrated the importance of *Nrob1* in spermatogenesis more so than in ovarian development.

PCSK1

We are only aware of a single case of IHH caused by a mutation in the prohormone convertase, subtilisin/kexin type, 1 (PCSK1) gene [125–127]. Another described patient died at 18 months. The affected female presented with extreme early-onset obesity, IHH, hypocortisolism, and abnormal glucose homeostasis. She had elevated plasma proinsulin and pro-opiomelanocortin (POMC) concentrations, and very low insulin levels [125–127]. Compound heterozygous PCSK1 mutations were demonstrated to have functional effects in vitro. The frame-

shift mutation predicted protein truncation, and the missense mutation prevented processing and lead to retention in the endoplasmic reticulum [125–127]. Because of the similarity of this proband to the *fat/fat* mouse, it appears that molecular defects in prohormone conversion may constitute a mechanism for obesity and endocrine disease common to humans and rodents.

Pituitary Genes

HESX1

A variety of genes involved in gonadotropin secretion and function may cause hypogonadotropic hypogonadism; however, they will only be briefly discussed here. HESX1 is a transcription factor and member of the paired-like homeobox genes, which contains repression domains thought to cause repression of transcription of target genes. The mouse ortholog, *Hesx1*, is expressed in the early forebrain, but later in development its expression is restricted to Rathke's pouch, which becomes the anterior pituitary gland [128]. Humans with either heterozygous (autosomal dominant) or homozygous (autosomal-recessive) HESX1 mutations may have septo-optic dysplasia (agenesis of corpus callosum, panhypopituitarism, and hypoplastic optic nerves) [128–135]. However, HESX1 mutations only account for a small percentage of those with SOD. Other HESX1 mutations have been found in isolated growth hormone deficiency [128–135]. HESX1 mutations may interfere with DNA binding [128] or the repression of target genes such as *PROP1* [133, 134].

PROP1

Like HESX1, *PROP1* is also a member of the paired-like homeobox genes. In contrast to HESX1 though, *PROP1* is thought to be both a transcriptional activator and repressor. *PROP1* mutations cause autosomal recessive combined pituitary hormone deficiency (CPHD), indicating its importance in anterior pituitary gland development. These patients may have deficiencies of GH, TSH, prolactin, gonadotropins, and ACTH [132, 135–140]. They typically demonstrate extreme short stature because of deficiencies of both GH and TSH. Although there have been many different *PROP1* mutations, the most common is a 2-bp frameshift deletion [132, 135–140]. No causative *PROP1* mutations were identified in 175 unrelated IHH/KS patients who did not manifest deficiencies of other pituitary hormones [140]. Mutations of mouse *Prop1* cause the Ames dwarf mouse, which has a similar phenotype to that of humans [141].

SOX2 and SOX3

Mutations in these two HMG box transcription factors have been found in humans with hypogonadotropic hypogonadism. Heterozygous *de novo* SOX2 mutations have been identified in patients with bilateral anophthalmia/microphthalmia, developmental delay, short stature, and male genital tract abnormalities. Recently, mice heterozygous for a targeted Sox2 disruption did not manifest these eye defects, but demonstrated abnormal anterior pituitary development with GH, TSH, and LH deficiency [142]. Eight of 235 (3.4%) patients with 'congenital hypothalamic-pituitary disorders' were found to have heterozygous SOX2 sequence variations, most of which were *de novo* [142]. Some mutations were nonsense or frameshift deletions or insertion, while others were missense mutations that were shown to impair function – either DNA binding, nuclear translocation, or transactivation. Patients with SOX2 mutations had eye defects, along with anterior pituitary hypofunction including IHH. However, there were also additional defects of the corpus callosum, hypothalamic hamartomas, sensorineural hearing loss, and esophageal atresia [142]. These findings in both mouse and human suggest that SOX2 is necessary for the normal development and function of the HPG axis.

Mutations in SOX3 cause an X-linked recessive form of pituitary deficiency. Several SOX3 mutations were reported, the first an in-frame 33-bp duplication in a family with X-linked mental retardation, facial abnormalities, and GH deficiency [143]. Another 686-kb duplication was identified in two siblings with variable degrees of hypopituitarism, corpus callosal abnormalities, anterior pituitary hypoplasia, an ectopic posterior pituitary, and an absent infundibulum [144]. Mental retardation was not present in these siblings. Corroborative findings have been identified in mice with targeted deletion of SOX3. The phenotype was that of impaired pituitary development and midline structures [145]. These pituitary and hypothalamic defects persisted postnatally.

LHX3 and LHX4

Both LHX3 and LHX4 are homeobox genes, which are transcription factors involved in anterior pituitary development. The LHX3 protein has two LIM motifs at the amino-terminus and a DNA-binding region in the carboxy-terminus. LHX3 mutations cause autosomal-recessive CPHD, while LHX4 mutations cause an autosomal-dominant form [132, 146, 147]. The phenotype of patients with LHX3 gene mutations consists of severe growth retardation (TSH and GH deficiency) and hypogonadotropic hypogonadism [132, 146, 147]. All pituitary hor-

mones (TSH, GH, prolactin, FSH, and LH) except ACTH are deficient. Of interest, severe restriction of cervical spine rotation with elevation and anteversion of the shoulders occurs in these patients, which is similar to what is seen with mouse *Lhx3* disruption. Although a family with GH, TSH, and ACTH deficiency due to a heterozygous mutation in the LHX4 gene has been described, the child was prepubertal, so gonadotropin levels were not investigated [148]. Recently, a second child was reported with a heterozygous LHX4 missense mutation [149]. Theoretically, LHX4 should also cause hypogonadotropic hypogonadism, but no pubertal aged children have yet been described.

Gonadotropin Genes (LHB and FSH)

Dimeric pituitary glycoprotein hormones consist of a common α -subunit encoded by the chorionic gonadotropin- α (CGA) gene and a specific β -subunit (TSH, hCG, FSH, and LH) encoded by the corresponding gene. Human CGA mutations have yet to be described, but hypothyroidism and hypogonadotropic hypogonadism would be expected. The human LHB/HCGB gene complex consists of a single LHB gene and six HCGB genes, which are highly polymorphic [150]. Several LHB mutations have now been identified. The extended phenotype, however, can be very variable and cause either hypergonadotropic or hypogonadotropic hypogonadism [151, 152]. The first LHB mutation (a homozygous missense mutation) was found in a male with delayed puberty, small testes, oligospermia, and low serum testosterone with elevated immunoreactive, but reduced biologically active LH levels [151]. If the mutation results in inability of the LH molecule to be processed and secreted, isolated LH deficiency will be present. In another male with a different missense mutation, LH was unable to be processed and secreted, so the phenotype was isolated LH deficiency [152]. This patient had a eunuchoid habitus, gynecomastia, small (8 ml) testes, undetectable LH, and elevated FSH (23 mIU/ml). Injections of hCG for a year increased testicular volume to 14 ml but improved sperm count only to 1,000/ml. Recently the first female with an LHB mutation was reported, and as predicted puberty was normal, but she was amenorrheic with infertility. The mutation was a homozygous splice mutant that was seen in this female and two affected males from their consanguineous family [153].

FSHB gene mutations have been identified in autosomal recessive isolated FSH deficiency in several females [154–156] and males [155, 157–159]. All mutations (missense, nonsense, or 2-bp deletion) in females have been

found in exon 3, all of which result in low immunoreactive and bioactive FSH levels probably by impairing dimer formation and secretion. Most females completely lack breast development and present with primary amenorrhea [154–156]. Estradiol levels are low (menopausal); FSH is undetectable or very low; and LH levels are elevated in females (LH may be normal to mildly elevated in males).

Although females with isolated FSH deficiency have elevated serum LH levels, they do not have hirsutism or hyperandrogenemia [154, 160, 161]. Since isolated FSH deficiency is associated with low androgens, this suggests that FSH plays an important role in ovarian androgen production, perhaps by inducing the androgen-dependent enzyme 17-hydroxylase activity, inhibin, growth factors, or increasing LH receptors [154, 160, 161].

New IHH/KS Candidate Genes

CHD7

Mutations in the human chromodomain helicase DNA-binding protein 7 (CHD7) gene have been shown to cause CHARGE syndrome, an autosomal-dominant or sporadic disorder consisting of coloboma of the eye, heart defects, choanal atresia, retardation of growth and development, genito-urinary anomalies, ear abnormalities (vestibular and auditory) [162]. This gene was initially identified using array comparative genomic hybridization (CGH) to identify a 2.3-Mb de novo microdeletion on chromosome 8q12 in 2 patients with CHARGE syndrome. One of these patients had an apparently balanced translocation reported previously [163]. All nine genes within the region were sequenced in 17 CHARGE syndrome patients without deletions, and 10 heterozygous CHD7 mutations (7 nonsense, 2 missense, and 1 potential splice mutant) were identified [162]. Of interest, CHD7 mutations occur in 60–80% of patients with CHARGE syndrome and span across the gene, but there currently appears to be no correlation between genotype and phenotype and most are de novo [162, 164–166]. Available data indicates that CHD7 appears to play an important role in the nucleus in binding to chromatin and regulating gene expression [162, 167].

Mutations in the mouse *Chd7* gene have been identified by ENU mutagenesis techniques in mice. Multiple alleles associated with vestibular disturbances (head bobbing and circling behavior) due to abnormal lateral semicircular canals clustered together on mouse chromosome 4 [168]. Upon DNA sequencing of the mouse *Chd7* gene, 9 heterozygous mutants were identified (6 nonsense and 3 splice mutants) confirming that *Chd7* was involved in

the mouse phenotype, similar to humans with CHARGE syndrome. Defects in these heterozygous mice included cleft palate, choanal atresia, cardiac septal defects, hemorrhage, prenatal death, vulvar and clitoral defects, and keratoconjunctivitis sicca [168].

Chd7 is expressed ubiquitously (by semiquantitative RT-PCR) in fetal and adult tissue, including the olfactory epithelium, eye, inner ear, and vascular system [162]. However, at E10.5 in the mouse, *Chd7* is selectively expressed in the forebrain, olfactory pit, optic vesicle, hindbrain, cardiac outflow tract, facio-acoustic preganglion, and mandibular portion of the first branchial arch [164]. In addition, loss of function in gene-trapped reporter mice resulted in embryonic lethality at E10.5 [169], which suggests that homozygous mutations might also be lethal. *Chd7* expression in this useful animal model was localized to the brain, retina, ear, craniofacial structures, pituitary, heart, and kidney at the expected time of endogenous *Chd7* mRNA expression (E10.5) [169].

Several studies implicate the CHD7 gene in IHH/KS, although the patients described have not been fully characterized. In a study of 14 children with CHARGE syndrome aged 6–18 years, all were found to be anosmic or hyposmic compared with controls [170]. In another study of CHARGE syndrome children, 19/20 affected boys had micropenis and/or cryptorchidism suggestive of IHH, although all were prepubertal [171]. Seven of 9 neonatal boys had low serum testosterone levels during the window period (<6 months of age) when testicular function can be determined. None of 7 females >12 years began puberty spontaneously and 5/7 had a subnormal FSH and LH responses to GnRH stimulation. Ten CHARGE syndrome patients had anosmia when tested by a smell test and all 18 having an MRI had abnormal olfactory bulbs. These findings are not conclusive, but nevertheless suggest that some patients with CHARGE syndrome could have an IHH/KS phenotype [171]. In fact, recently a female affected with CHARGE syndrome due to a CHD7 mutation had a KS-like phenotype [172]. These findings indicated that perhaps IHH/KS could be a milder allelic variant of CHARGE syndrome, which warrants further study.

NELF

Nasal embryonic LHRH factor (NELF) was cloned and characterized from migrating GnRH neurons using differential screening techniques [173]. A knockout mouse has not yet been published at the time of this writing. High levels of mRNA and protein expression were

found in the forebrain, olfactory epithelium, and olfactory pit of embryos with maximal expression between E12.5 and E14.5 in the olfactory epithelium and olfactory pit [173]. NELF protein expression was demonstrated in the cell soma and processes and appeared to be on the surface of the neurons. Since NELF is present on both olfactory and GnRH neurons, these authors suggested that NELF may serve as a common guidance cue for olfactory axon projections and subsequent migration of GnRH neurons [173].

When nasal explants were treated with antisense oligonucleotides, NELF protein decreased by 60%, GnRH neuron fiber complexity and length decreased, and there was a reduction in the number of GnRH neurons in the periphery of the explant. These findings suggested that NELF may play an important role in GnRH neuron migration, puberty, and reproduction. The human ortholog was cloned by Miura et al. [174] and, although they identified one heterozygous missense mutation, no functional analysis was performed [174]. Recently, the coexistence of a NELF mutation and an FGFR1 mutation was reported, which together produced IHH [175]. These findings in mice and humans indicate that NELF certainly has the potential to be an important candidate gene for IHH/KS.

PROK2 and PROKR2

Since mice lacking the prokineticin receptor (*Prokr2*) demonstrate impaired development of the olfactory bulbs and deficient GnRH neurons [176], genes for the both the ligand prokineticin 2 (*PROK2*) and its receptor (*PROKR2*) have been studied in patients with IHH [177]. Although putative mutations were identified in both the ligand and the G-protein-coupled receptor, the functional significance of these genes in IHH/KS awaits confirmation by additional studies.

Conclusions

GnRH and olfactory neurons migrate from the olfactory placode region into the brain. GnRH neurons, along with a branch of the vomeronasal nerve, finally reside in the hypothalamus, where they secrete pulsatile GnRH which controls the neuroendocrine functions of reproduction. Impairment of both GnRH and olfactory neuron migration results in KS, the combination of IHH and anosmia, while some patients exhibit normosmic IHH. Although long thought to be an irreversible process, it now appears that LH pulsatility may be restored in some patients when treatment is discontinued. Mutations in the *KAL1* and *FGFR1* genes constitute the only known molecular etiology for patients with KS, but *FGFR1* mutations also result in normosmic IHH.

Mutations in a variety of other genes also occur in normosmic IHH, most notably the *GNRHR*. Although not as common, the *GPR54* and *LEP/LEPR* genes play significant physiologic roles in normal puberty. A number of pituitary genes also possess mutations in patients with pituitary deficiency that includes hypogonadotropic hypogonadism. Finally, several new genes – *CHD7*, *NELF*, *PROK2*, and *PROKR2* – make excellent candidate genes for IHH/KS since they are expressed in GnRH and olfactory neurons. Digenic disease has recently been reported, and it will be interesting to further dissect the molecular basis of disorders of puberty and reproduction. Candidate gene screening and positional cloning utilizing balanced chromosomal rearrangements or affected consanguineous families will reveal additional important genes involved in the pathogenesis of IHH/KS.

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