HHS Public Access

Author manuscript

Metabolism. Author manuscript; available in PMC 2018 September 01.

Published in final edited form as:

Metabolism. 2018 September ; 86: 124–134. doi:10.1016/j.metabol.2017.10.012.

Kallmann syndrome: phenotype and genotype of hypogonadotropic hypogonadism

Maria I. Stamou[a,b,c,*,1] and **Neoklis A. Georgopoulos**[b]

[a]Harvard Reproductive Sciences Center, Massachusetts General Hospital, Boston, MA, USA

[b]University of Patras Medical School, University Hospital, Department of Obstetrics and Gynecology, Division of Reproductive Endocrinology, Rion, Patras, Achaia, Greece

[c]Mount Auburn Hospital, Harvard Medical School Teaching Hospital, Cambridge, MA, USA

Abstract

Isolated Gonadotropin-Releasing Hormone (GnRH) Deficiency (IGD) IGD is a genetically and clinically heterogeneous disorder. Mutations in many different genes are able to explain ~40% of the causes of IGD, with the rest of cases remaining genetically uncharacterized. While most mutations are inherited in X-linked, autosomal dominant, or autosomal recessive pattern, several IGD genes are shown to interact with each other in an oligogenic manner. In addition, while the genes involved in the pathogenesis of IGD act on either neurodevelopmental or neuroendocrine pathways, a subset of genes are involved in both pathways, acting as “overlap genes”. Thus, some IGD genes play the role of the modifier genes or “second hits”, providing an explanation for incomplete penetrance and variable expressivity associated with some IGD mutations.

The clinical spectrum of IGD includes a variety of disorders including Kallmann Syndrome (KS), i.e. hypogonadotropic hypogonadism with anosmia, and its normosmic variation normosmic idiopathic hypogonadotropic hypogonadism (nIHH), which represent the most severe aspects of the disorder. Apart from these disorders, there are also “milder” and more common reproductive diseases associated with IGD, including hypothalamic amenorrhea (HA), constitutional delay of puberty (CDP) and adult-onset hypogonadotropic hypogonadism (AHH). Interestingly, neurodeveloplmental genes are associated with the KS form of IGD, due to the topographical link between the GnRH neurons and the olfactory placode. On the other hand, neuroendocrine genes are mostly linked to nIHH. However, a great deal of clinical and genetic overlap characterizes the spectrum of the IGD disorders. IGD is also characterized by a wide variety of non-reproductive features, including midline facial defects such as cleft lip and/or palate, renal agenesis, short metacarpals and other bone abnormalities, hearing loss, synkinesia, eye movement abnormalities, poor balance due to cerebellar ataxia, etc. Therefore, genetic screening should be offered in patients with IGD, as it can provide valuable information for genetic counseling and further understanding of IGD.

[*]*Corresponding author at*: Mount Auburn Hospital, Harvard Medical School Teaching Hospital, Cambridge, MA, USA. mstamou@mah.harvard.edu (M.I. Stamou).

[1]Postal address: 330 Mount Auburn Street, Cambridge, MA, USA.

Declaration of Interest

No conflict of interest.

Keywords

Kallmann syndrome; Genetics; Hypogonadotropic hypogonadism; Reproduction

1. Introduction

Isolated Gonadotropin-Releasing Hormone (GnRH) Deficiency (IGD) is a rare heritable disorder that is clinically and genetically heterogeneous with an incidence of 1:125,000 in females and 1:30,000 in males [1]. IGD spans a broad clinical spectrum consisting mainly of Kallmann Syndrome (KS), i.e. hypogonadotropic hypogonadism with anosmia, and its normosmic variation normosmic idiopathic hypogonadotropic hypogonadism (nIHH). In both KS and nIHH patients the rest of the hypothalamic and pituitary hormones as well as the radiographic appearance of hypothalamic-pituitary region are typically normal. Apart from those 2 phenotypic presentations that represent the most severe aspects of the disorder, there is a plethora of "milder" common reproductive diseases associated with the hypothalamic-pituitary-gonadal (HPG) axis including hypothalamic amenorrhea (HA) [2], constitutional delay of puberty (CDP) [3], and adult-onset hypodonadotropic hypogonadism (AHH) [4].

Close analysis of IGD pedigrees often reveals an X-linked, autosomal recessive, or autosomal dominant inheritance pattern [5–9]. In addition to these Mendelian modes of inheritance, an even more complex genetic architecture for IGD (often referred as oligogenicity, occurring in 10–15% of IGD cases) has now been documented, wherein mutations in two or more IGD associated genes are found in a single case [10–12]. This genetic complexity is very well-studied and to date there are almost 35 genes implicated in IGD [13], with the majority of the IGD patients still remaining genetically uncharacterized. These genes regulate neurodevelopmental and neuroendocrine IGD pathways causing KS and nIHH, respectively but with a great deal of overlap between the genetic causes that contribute to both aspects of the disorder. Interestingly, genetic association of IGD and common, related reproductive diseases has been reported. In particular known IGD genes are found to be mutated in patients with HA and CDP. On the other hand, genome-wide association studies for the age of menarche and menopause are surfacing genetic loci in close proximity to known IGD genes [14–17].

1.1. Pathophysiology of GnRH Development and Function

Reproductive function in humans is mainly controlled by ~1200–1500 GnRH neurons. During embryogenesis immature GnRH cells migrate from the olfactory epithelium, through the cribriform plate, into the developing olfactory bulb, and then through the forebrain to their final position in the hypothalamus [18]. These unique neurons have either an olfactory place/ectodermal or a neural crest cell origin [19–27]. At the time of puberty, these neurons coordinately secrete GnRH in a pulsatile way. This pulsatile pattern of GnRH secretion is the key stimulator for the production of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) by the pituitary. In turn, LH and FSH act on the gonads with 2 different goals: (i) the secretion of sex steroids, i.e. testosterone in men and estrogen in women, both important for the development of secondary sexual characteristics, and (ii) the production of

germ cells. In both sexes, these GnRH neurons are fully active and secreting GnRH during the neonatal period [28]. However, this GnRH secretory activity becomes quiescent in childhood and, mysteriously, reawakens again during adolescence, marking the onset of puberty. Defects in the development of GnRH neurons or their secretory function result in isolated GnRH deficiency (IGD), and thus disruption of normal puberty.

1.2. Genotypic Characteristics of KS

IGD is caused by rare sequence variants (RSVs) in a number of different genes and to-date, ~40% of patients have a genetic mutation that is identifiable [13]. As shown in Table 1, while some genes primarily cause KS, others cause nIHH only, while others cause both forms of IGD. Mutations in genes that disrupt the development and migration of GnRH neurons cause KS, and such genes include *KAL1* - Kallmann 1, *NSMF* - NMDA Receptor Synaptonuclear Signaling And Neuronal Migration Factor, *FGFR1* - Fibroblast Growth Factor Receptor 1, *FGF8* –Fibroblast Growth Factor 8, *FGF17* – Fibroblast Growth Factor 17, *IL17RD* – Interleukin 17 Receptor D, *PROK2* – Prokineticin 2, *PROKR2* – Prokineticin Receptor 2, *HS6ST1* – Heparin Sulfate 6 O Sulfutransferase, *CHD7* – Chromodomain Helicase DNA Binding Protein 7, *WDR11* – WD Repeat-Containing Protein 11, *SEMA3A* – Semaphorin 3A, *TUBB3* – Tubulin Beta 3, *SOX10* – SRY Box 10 and many more (as shown in Table 1) [6,29].

On the other hand, genes that interfere with the neuroendocrine physiology of the normal secretion of GnRH (*GNRH1* – GnRH 1, *KISS1* – Kisspeptin 1, *KISS1R (GPR54)* – Kisspeptin 1 Receptor, *TAC3* – Tachykinin 3, *TACR3* – Tachykinin Receptor 3, *LEP* - Leptin, *LEPR* – Leptin Receptor) or its action on the pituitary (*GNRHR* – GnRH Receptor), cause nIHH [6,7]. The majority of the genes are considered “overlap genes” (i.e. the ones that are found to be disrupted in both KS and nIHH patients) and these (so far) include *NSMF*, *FGFR1, FGF8, FGF17, IL17RD, PROK2, PROKR2, HS6ST1, CHD7, WDR11* and *SEMA3A*. Presumably, these genes may have multiple roles in GnRH biology including both GnRH neuronal migration and their normal secretory function, although for many genes this remains to be examined [29].

2. Neurodevelopmental Genes

2.1. KAL1

The first gene found to be responsible for KS is localized to the distal portion of X chromosome (Xp22.3). It was discovered by studying patients with a “contiguous gene syndrome” causing short stature, chondrodysplasia punctata, intellectual disability, icthyosis, and KS. By mapping the genes within this large deletion, the *KAL1* gene was identified as the cause of KS. The protein encoded by *KAL1*, anosmin 1, is associated with neural cell adhesion and axonal migration, linking a CNS migratory defect to the phenotype expressed in KS patients [30]. In addition, anosmin is expressed in many other tissues including the digestive, respiratory, urogenital, cardiovascular, integumentary, skeletal and muscular system,and the placodal derivatives [31]. This robust expression in a variety of tissues could explain the variable phenotypes that are expressed by patients with KS. For example, the

expression of *KAL1* in the mesonephric duct and the tubules, is a clear genotypic-phenotypic link in *KAL1* carriers with KS and unilateral renal agenesis [32–34].

2.2. FGF8, FGFR1, FGF17, IL17RD, DUSP6, SPRY4, GLCE and FLRT3

Using an IGD patient with a different chromosomal breakpoint on 8p11.2-p11.1, *FGFR1*, a gene encoding Fibroblast Growth Factor Receptor 1, was identified as a cause of KS [35]. Since then, a large number of mutations in this gene have been uncovered as the cause of both KS and nIHH [36,37]. Although there are 23 known FGF ligands, using the crystallographic modeling information of these ligands and by studying a single *FGFR1* mutation, FGF8 was identified as the ligand responsible for GnRH neuronal migration. And mutations in *FGF8* were discovered in IGD patients [38]. More recently, using protein-protein interactions, a number of other genes have been discovered, including *FGF17*, *IL17RD*, *DUSP6*, *SPRY4*, *GLCE*, and *FLRT3*, with *FGF17* and *IL17RD* having an important role in GnRH development [39].

2.3. PROK2 and PROKR2

Following the demonstration of deletions of Prok2 (prokineticin 2) and Prokr2 (prokineticin 2 receptor) as genetic causes of KS in mice, mutations in humans (*PROK2* and *PROKR2*) were identified to cause both KS and nIHH [40,41]. Both genes are critical regulators of both GnRH neuronal development as well as the GnRH release. *PROK2* is expressed in various sites in the mouse brain, including the suprachiasmatic nucleus, the arcuate nucleus and the medial preoptic area of the hypothalamus, whereas *PROKR2* is highly expressed in the olfactory ventricle and subventricular zone of the lateral ventricle, both of which serve as origins for neuronal precursors [42]. Both molecules are associated with the neurogenesis of the olfactory bulbs and the migration of olfactory neuronal cells [43,44].

2.4. NSMF

The NMDA receptor synaptonuclear signaling and neuronal migration factor, *NSMF*, has been shown to function as a guidance molecule for olfactory axon projections and neurophilic migration of GnRH cells in mice. Mutations in *NSMF* have been discovered in IGD patients (both KS and nIHH) primarily in an oligogenic inheritance pattern. The role of *NSMF* in the GnRH neurodevelopmental pathway was first tested in mouse nasal explants, where the protein was robustly expressed. Treatment with *NSMF* antisense oligonucleotides resulted in reduction of axonal outgrowth and movement, suggesting a role of *NSMF* in the defective migration of the olfactory neurons in KS patients [45,46].

2.5. WDR11

The *WDR11* gene encodes for WD Repeat Containing Protein 11. Heterozygous mutations in *WDR11* have been linked to IGD. While both KS and nIHH subjects harbored variants in *WDR11*, murine studies show interaction of *WDR11* with *EMX1* (a homeodomain transcription factor in olfactory neuronal development), thus accounting for its implication in KS. The precise biologic role of *WDR11* in neurodevelopmental regulation of GnRH is yet to be established [47].

2.6. HS6ST1

Mutations in *HS6ST1* gene, encoding heparan sulfate (HS) 6-O-sulfotransferase, a member if heparan sulfate polysaccharides were also identified as an oligogenic cause of IGD (both KS and nIHH) [48]. *HS6ST1* catalyzes the transfer of sulfate from 3-prime-phosphoadenosine 5-prime-phosphosulfate to position 6 of the N-sulfoglucosamine residue of heparan sulfate and plays an important role in cell-cell communication and neuronal development. Genetics experiments in the worm, (*C. elegans*) have shown that *HS6ST1* specifically regulates neural branching in vivo acting togehter with other IHH-associated genes, such as *KAL1, FGFR1* and *FGF8*. These findings are consistent with a model in which anosmin-1 can act as a modulatory co-ligand with *FGF8* to activate the *FGFR1* receptor in an HS-dependent manner [48].

2.7. SEMA3A and SEMA3E

In 2012, mutations as well as partial deletions in *SEMA3A*, encoding a secreted axonal guidance molecule, semaphorin 3A, were identified in ~6% of KS patients [49,50]. Semaphorin 3A acts by activating the neuropilin-plexin-A1 holoreceptor complex. It functions as an axonal repulsive cue to the axonal growth cone during embryonic development. Supportive data from both murine deletions of Sema3a as well as mice with specific mutation in the semaphorin binding domain of its receptor show abnormal development of the peripheral olfactory system and defective embryonic migration of the neuroendocrine GnRH cells to the basal forebrain. A more recent study of 2 brothers with KS identified mutations in another family member of Semaphorin 3 class, *SEMA3E*. Additional studies in animal models confirmed that mutations in this gene can cause features similar to those presented in KS, especially in oligenicity with *CHD7* [51].

2.8. CHD7, TUBB3 and SOX10

Mutations in the gene coding for Chromodomain helicase DNA binding protein 7-*CHD7* cause CHARGE syndrome (eye coloboma, heart anomalies, choanal atresia, growth and developmental retardation, genitourinary anomalies and ear abnormalities) (OMIM #214800). The “G” in CHARGE is related to hypogonadism occurring secondary to IGD. Milder allelic variants in *CHD7* have been linked to a non-syndromic presentation of IGD (both KS and nIHH) [52–54]. IGD patients with *CHD7* mutations may also have additional CHARGE related features, such as hearing loss [55]. *CHD7*, an ATP-dependent chromatin remodeler plays an important role in the formation of a multipotent migratory neural crest (NC) cell population that is crucial for the development of craniofacial bones and cartilage, the peripheral nervous system, pigmentation, and cardiac structures [56,57]. Importantly, *CHD7* mRNA is expressed in both migratory and postmigratory GnRH neuronal cell lines as well as in the hypothalamus, pituitary, and olfactory bulb in the rat during the appropriate timing for GnRH migration [47]. Additionally, *CHD7* regulates genes involved in neural crest cell guidance, including *SEMA3A*, which act as an important regulator of olfactory and cortical neuronal guidance as well as GnRH neuronal migration [58].

2.9. SOX10 and TUBB3

Other genes associated with syndromic forms of IGD, such as *SOX10* and *TUBB3*, have been associated with a disruption of neural crest (NC) cell migration, indicating their role in the alternate GnRH neuronal migratory pathway [59,60]. Importantly, IGD patients carrying mutations in such genes display additional phenotypic characteristics that could be attributed to defective development of neuronal crest cells, such as craniofacial defects, midline and retinal abnormalities, as well as cardiac and inner ear defects. In particular, *SOX10*, a marker of NC-derived olfactory neuronal cells, has been identified as the genetic cause for the association of IGD with Hirshsprung's disease [61]. Moreover, *TUBB3*, a member of the β-tubulin family that is known as a neuronal and melanocyte marker, is disrupted in patients with KS and cranial as well as peripheral neuropathy, suggesting its potential role in the NC cell and GnRH neuronal migratory pathways [60].

2.10. POLR3A, POLR3B, OTUD4, RNF216, STUB1 and PNPLA6

Advanced techniques including whole genome sequencing have been used to identify a variety of new genes that cause hypogonadotropic hypogonadism associated with cerebellar ataxia, also known as Gordon Holmes Syndrome. These include OUT Domain Containing Protein 4 -*OTUD4*, Ring Finger Protein 216 - *RNF216*, Polymerase III RNA Subunit A - *POLR3A*, Polymerase III RNA Subunit B - *POLR3B*, Patatin-Like Phospholipase Domain-Containing Protein 6 - *PNPLA6*, and Stip1 Homologous And U Box Containing Protein 1 - *STUB1*, with several animal studies proving their functionality [62–65].

2.11. FEZF1

Using next generation sequencing a recent analysis of a consanguineous family of Kurdish origin revealed a gene called Fez Family Zinc Finger Protein 1 - *FEZF1*, that was found to be the cause for the expression of the phenotype in this family [66].

2.11.1. DMXL2—Finally, a new syndrome in 3 brothers, which involves the hypothalamic-pituitary-gonadal axis, central hypothyroidism, peripheral demyelinating sensorimotor polyneuropathy, mental retardation, and profound hypoglycemia progressing to a non-autoimmune insulin-dependent diabetes mellitus was genetically explained by mutations found in the gene DMX like 2 - *DMXL2* [67].

3. Neuroendocrine Genes

3.1. GNRH1 and GNRHR

Both *GNRH1* and its receptor *GNRHR* are obvious candidate genes to cause IGD. *GNRHR* mutations are relatively common and cause the nIHH form of IGD. Studies in patients with *GNRHR* mutations reveal a heterogeneous clinical presentation, with both autosomal recessive and oligogenic inheritance patterns of inheritance [68]. After several years of investigation, *GNRH1* mutations were eventually shown to be a cause of GnRH deficiency in 2009. Mutations in GNRH1 are extremely rare and were only identified after genetic studies were done in over 400 patients with IGD [69].

3.2. KISS1R (GPR54) and KISS1

In 2003, two independent groups utilizing endogamous pedigrees identified autosomal recessive mutations in *KISS1R* (formerly called *GPR54*) as the cause of nIHH form of IGD [70,71]. The *KISS1R* encodes the kisspeptin receptor (a cognate G-protein-couple receptor) for the ligand, kisspeptin. Kisspeptin is a secreted neuropeptide and it is now well-established as an upstream regulator of the GnRH neurons. Mutations in the gene *KISS1* encoding kisspeptin itself, are also found to underlie autosomal recessive nIHH [72]. Both *KISS1* and *KISS1R* mutations affect the secretion of GnRH rather than the migration of GnRH neurons, thus resulting in nIHH exclusively. Administration of kisspeptin analogs can stimulate gonadotropin secretion via activation of hypothalamic GnRH as shown in rats, mice and humans, highlighting its potential role as a therapeutic target [73–80]. Additionally, *Kiss1* and its receptor are expressed in hypothalamus of male and female rats and mice and this expression is subjected to the negative feedback of sex steroids during the estrus cycle supporting the role of the KISS family in the neuroendocrine regulation of the gonadotropin secretion [81,82]. Importantly, *Kiss1r* is localized in a large proportion of murine GnRH neurons throughout embryonic as well as pubertal development [83,84]. These human and mouse genetic observations, as well as supportive data from other species, now confirm that kisspeptin signaling is the most robust stimulator of GnRH secretion known currently.

3.3. TAC3 and TACR3

Using homozygosity mapping in consanguineous pedigrees, two novel genes involved in tachykinin signaling, *TAC3* (encoding neurokinin B) and its receptor (*TACR3*) were identified as causes of nIHH [85]. Subsequently, mutations in these two genes were also identified in non-endogamous IGD patients and it was shown that the neurokinin pathway plays an important role both in ‘mini-puberty’ as well as the GnRH activation in puberty. However, longitudinal studies have revealed that several subjects with *TAC3/TACR3* mutations eventually reverse their GnRH deficiency in adulthood, suggesting that this pathway may be dispensable for adult reproductive function [86]. Interestingly, a few years before the identification of the *TACR3* and *TAC3* as causative genes for the expression of IGD, neurokinin B was found to be co-expressed with kisspeptin and along with dynorphin form a subset of neurons, referred to as KNDy neurons, that are located in the arcuate nucleus and regulate the GnRH neuronal function [87–89].

3.4. NROB1

The nuclear receptor subfamily 0, group B, member 1 (*NR0B1*) gene is a neuroendocrine pleiotropic IGD gene. It’s discovery explained the paradox of the well-established association between the X-linked form of congenital adrenal hypoplasia and hypogonadotropic hypogonadism via its previously undescribed role in the development of the hypothalamic-pituitary axis [90].

3.5. LEP/LEPR

Although IGD is only occasionally associated with morbid obesity, genetic defects in leptin (*LEP*) and the leptin receptor (*LEPR*) genes, as well as mutations in the proprotein

convertase subtilisin/kexin type 1 (*PCSK1*) gene, have been found in IGD patients with an obesity phenotype [91–95]. In addition to the steroid hormonal feedback that is essential for the regulation of GnRH neurons, energy balance, body weight, and food intake have also been shown to serve as initiating factors for puberty. Interestingly, leptin treatment restores hypogonadism in leptin-deficient mice due to starvation [96] and also stimulates the initiation of puberty in normal female mice [97]. Additionally, leptin regulates the HPG axis in humans, as shown in studies where healthy adults as well as females with hypothalamic amenorrhea were analyzed [98]. As illustrated in Fig. 1, after the synthesis and secretion of leptin form the adipose tissue into the circulation, it acts at the level of hypothalamus in three different ways, including: (i) suppression of NPY (neuropeptide-Y) neuronal activity, which reduces the stimulatory drive on food intake and decreases NPY inhibition of kisspeptin cell bodies (ii) stimulation of a-MSH (melanocyte stimulating hormone) via POMC (proopiomelanocortin), which functions to suppress food intake and alter growth, and (iii) direct interaction with subpopulations of kisspeptin neurons to further increase the stimulatory drive on GnRH (gonadotropin-releasing hormone) release and gonadotropin secretion from the pituitary gland [99]. Any fluctuation in energy balance changes leptin secretion and alters these pathways [100]. Even though most experiments have shown an increase in the *Kiss1* mRNA expression in the arcuate nucleus after leptin administration [99], selective deletion of leptin receptor in kisspeptin neurons of mice was not shown to impair their ability to reach puberty nor their subsequent fertility [101].

4. Clinical Presentation

4.1. Reproductive Features of IGD

The clinical hallmark of IGD is the failure of onset of puberty. Hypogonadotropic hypogonadism is characterized by reduced blood levels of the sex hormone levels as well as gonadotropins (LH and FSH) and infertility. In males, the onset of normal pubertal development is linked to testicular enlargement that is then followed by penile growth and the appearance of pubic hair. Affected men complain of absence of secondary sexual characteristics and a delayed growth spurt in comparison to their peers. In addition, low libido and poor sexual function may also be present. Gynecomastia may also be rarely seen in these subjects although this more typically occurs during treatment and is often transient (see below) [102].

Physical examinations in these patients usually confirms incomplete sexual maturation (e.g. prepubertal testicular volume [< 4 ml]), decreased muscle mass and eunuchoid body habitus. Although IGD in males is typically diagnosed at puberty, this diagnosis can be made in infancy due to micropenis/microphallus and/or cryptorchidism. As mentioned earlier, pulsatile GnRH secretion and evidence of a normal reproductive axis occurs during the neonatal period [28]. Hence, timely biochemical testing (i.e. low gonadotropin levels) during the first months of life may also confirm the presence of hypogonadism. However, if this brief developmental window of diagnostic testing is missed, a definite diagnostic confirmation may have to wait until the expected time of puberty.

In females, the first sign of normal puberty is the onset of thelarche, followed by a growth spurt, the appearance of pubic hair growth, and then only later, the menarche. IGD females

typically present with an absence of breast development, an attenuated growth spurt, decreased pubic hair growth and primary amenorrhea. However, some females may also exhibit signs of partial puberty and secondary amonerrhea. Clinical exam in IGD females usually confirms their immature sexual characteristics and enuchoid habitus. It is important to note that development of pubic hair can be normal in both sexes as it is controlled by secretion of androgens from the adrenal glands, i.e. adrenarche, which is unaffected in IGD subjects [103]. Importantly, detailed olfactory function testing is recommended in all patients with hypogonadotropic hypogonadism to guide us classify the disease to KS vs. nIHH as well as brain imaging and other biochemical testing to rule out any other causes of HH such as structural lesions, other hormonal changes (such as hyperprolactenemia) and genetic disorders such as hemochromatosis.

4.2. Non-reproductive Features of IGD

The majority of IGD subjects also exhibit a spectrum of other non-reproductive features and these features may offer clues to the underlying genetic etiology of IGD. Commonly recognized non-reproductive features that may be present in IGD subjects include: Midline facial defects such as cleft lip and/or palate, renal agenesis, short metacarpals, hearing loss, synkinesia, eye movement abnormalities, poor balance due to cerebellar ataxia, scoliosis and many more. Such non-reproductive phenotypic characteristics may serve as tools to prioritize the genetic testing in patients with IGD. In a study by Costa-Barbosa and colleagues [34], males carrying *KAL1* mutations displayed the most severe reproductive phenotype when complared with non-*KAL1* probands. Additionally, synkinesia was enriched but not unique to patients with *KAL1* RSVs and dental agenesis and digital bone abnormalities were enriched in patients with RSVs in the *FGF8/FGFR1* signaling pathway compared with all other gene groups combined. Hearing loss marked the probands with *CHD7* RSVs. Surprisingly, renal agenesis and cleft lip/palate did not emerge as statistically significant phenotypic predictors.

In addition, various multisystem disorders with overlapping features of KS/nIHH have been reported (see above). These include: CHARGE Syndrome linked to *CHD7*, adrenal hypoplasia congenita (AHC) (due to *NROB1* RSVs), congenital obesity syndromes (due to *LEP/LEPR* mutations), Bartlet-Biedl Syndrome (several genes), Moebius Syndrome (*TUBB3*), Gordon Holmes syndrome and cerebellar ataxia (*POLR3A, POLR3B, OTUD4, RNF216, STUB1* and *PNPLA6*), Hirschprung's disease (*SOX10*) as well as central hypothyroidism, peripheral sensorimotor polyneuropathy, mental retardation and non-autoimmune insulin-dependent diabetes mellitus (*DMXL2*).

4.3. Reversal of IGD

Even though IGD is a long standing disease, 20% of the IGD patients have been found to reverse at some point during their adult life [104]. The exact mechanism of reversal of this phenotype is still unclear. However, rare variants in *TAC3* and *TACR3* have been associated with this phenomenon and such genetic association could serve as a possible explanation for its patholhysiology [86]. Thus, genetic screening is recommended in all IGD patients as well as a "treatment holiday" for testing the reversibility of their disease.

5. Reproductive Disorders Associated with Igd

Apart from KS and nIHH that represent the most severe phenotypes of IGD there is a broad spectrum of IGD related diseases. Apart from the genetic overlap between KS and nIHH a common genetic background is shared between more common reproductive disorders and IGD.

5.1. Hypothalamic Amenorrhea

Functional hypothalamic amenorrhea (HA) is a reversible form of GnRH deficiency commonly triggered by stressors such as excessive exercise, nutritional deficits or psychological distress. This defect is usually reversible once the stressor no longer exists, although some patients require a long term hormonal replacement treatment. A common genetic basis between IGD and HA has been described with 7 out of 55 HA patients found to carry heterozygous rare sequence variants (RSVs) in genes 5 known IGD genes including *FGFR1, PROKR2, GNHR, GNRHR* and *KAL1*, with the RSVs in the first 4 genes being functionally validated [2].

5.2. Delayed Puberty

Constitutional delayed of puberty (CDP) is defined as the lack of sexual maturation at an age >2 SDs above the mean for a given population and it is self limited. In details it is characterized by (i) absence of spontaneous thelarche by the age of 13 yo and spontaneous menarche by the age of 15 yo in girls and spontaneous testicular growth by the age of 14 yo in boys (ii) spontaneous pubertal development by the age of 18 (iii) evidence of normal rate of pubertal progression (iv) absence of identifiable underlying causes of delayed puberty. One major difference between CDP and reversal of IGD is that the onset of puberty in CDP even though delays occurs before the age of 18yo in contrast to IGD. CDP is highly heritable with 50–80% of CDP patients having a positive family history of the same disease. The association between IGD and CDP is suggested by the fact that ~10% of IHH patients have relatives with CDP. In a recent study by Zhu et al. [3], IGD subjects shared their rare variant with the CDP family members in 53% compared to the unaffected relatives. Additionally, when a cohort of CDP patients was studied several RSVs were found in 5 out of 13 IGD studied with *TAC3* and *IL17RD* found to be enriched in the CDP cohort compared to controls. Interestingly, *TACR3* has also been associated with variation in pubertal timing [105].

5.3. Adult-Onset Hypogonadotropic Hypogonadism

In contrast to congenital IGD, Adult-Onset Hypogonadotropic Hypogonadism (AHH) is a rare form of IGD presenting in otherwise healthy adult males after completion of normal puberty and often proven fertility. AHH can result from different causes including: anatomic etiologies, infiltrative diseases, pituitary masses including adenomas, craniopharyngiomas and other CNS tumors. However, most of these causes can be ruled out in patients with AHH. In a long term detailed clinical follow up study of a small cohort of AHH men, the condition was proven to be non-reversible and long standing. A few RSVs in genes such as *GNRHR, FGF8* and *PROKR2* have been reported [4]. However, no systematic genetic

screening of AHH patients has been conducted so far. Thus, further studies are required to prove the genetic associated between IGD and AHH.

5.4. Clinical Implications of Genetic Screening in IGD

While next generation sequencing has been improving and becoming clinically available, opportunities for genetic screening in patients with rare diseases are emerging. Genetic screening in patients with IGD is very important for the following reasons: (i) providing with valuable genetic counselining for other family members or future offspring, and (ii) providing with supportive data that are important for the prognosis of the disease. For example, as mentioned earlier, 20% of the IGD patients have been found to reverse their phenotype at some point during their adult life [104]. Direct genetic association has been made with the genes of the tachykinin family. Thus, discovering loss of function mutations (LoF) in *TAC3* and *TACR3* in nIHH patients can serve as a risk stratification factor for the prognosis of the disease in such patients. As the number of genes discovered increases and functional validation of mutations is being established, such clinical-genetic correlations will be feasible and serve as a guidance for further management of IGD patients.

Given that financial limitations can emerge when it comes to genetic screening, selecting genes for targeted screening could be considered. Thus, if WES or WGS is not available at certain centers, targeted genetic screening can be utilized and non-reproductive features of IGD patients can be used as a guideness for the gene selection. As mentioned earlier, in the study by Costa-Barbosa and colleagues [34], severe reproductive phenotype and synkineisa has been linked with *KAL1*, digital and skeletal deformities with the *FGF* family and hearing loss with *CHD7*. In addition, any of the genes implicated in the syndromic forms of IGD should be prioritized first, when the patients express any of the syndromic non-reproductive features in their phenotype.

Finally, since the diagnosis of IGD in the neonatal period is mainly dependent on the timely biochemical testing during the first months of life (and if missed has to wait until the time of puberty), the increasing knowledge of the genetic basis of this condition may enable confirmation by specific genetic testing in the future.

6. Summary

In conclusion, IGD is caused by a large number of mutations in many different genes, which now explain ~40% of the genetic causes of the disorder. While most are inherited in a Mendelian pattern, several of these genes are shown to interact in an oligogenic manner and the majority of then act in both neurodevelopmental and neuroendocrine pathways. Some of them also play the role of the modifier genes. Such genetic complexity can explain the incomplete penetrance and variable expressivity that this disease is characterized by.

IGD genes can be divided into 2 broad categories: the neurodevelopemtnal and the neuroendocrine. Importantly, the majority of the genes described are considered “overlap genes” i.e. the ones that are found to be disrupted in both KS and nIHH carriers. Presumably, these genes may have multiple roles in GnRH biology including both migration and their normal secretory function, although for many genes this remains to be examined.

Finally, apart from the genetic overlap between KS and nIHH, genetic overlap is now starting to arise between IGD and common reproductive disorders, highlighting the great complexity of the GnRH genetic architecture.

Acknowledgments

Dr. Maria I Stamou would like to acknowledge Alexander S. Onasis Foundation.

Funding

This article did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Abbreviations

GnRH	Gonadotropin Releasing Hormone
KS	Kallmann Syndrome
nIHH	normosmic idiopathic hypogonadotropic hypogonadism
IGD	isolated GnRH deficiency
LH	luteinizing hormone
FSH	follicle-stimulating hormone
CDP	constitutional delay of puberty
HA	hypothalamic amenorrhea
AHH	adult-onset hypogonadotropic hypogonadism

References

1. Laitinen EM, Vaaralahti K, Tommiska J, Eklund E, Tervaniemi M, Valanne L, et al. Incidence, phenotypic features and molecular genetics of Kallmann syndrome in Finland. Orphanet J Rare Dis. 2011; 6:41. [PubMed: 21682876]
2. Caronia LM, Martin C, Welt CK, Sykiotis GP, Quinton R, Thambundit A, et al. A genetic basis for functional hypothalamic amenorrhea. N Engl J Med. 2011; 364(3):215–25. [PubMed: 21247312]
3. Zhu J, Choa RE, Guo MH, Plummer L, Buck C, Palmert MR, et al. A shared genetic basis for self-limited delayed puberty and idiopathic hypogonadotropic hypogonadism. J Clin Endocrinol Metab. 2015; 100(4):E646–4. [PubMed: 25636053]
4. Dwyer AA, Hayes FJ, Plummer L, Pitteloud N, Crowley WF Jr. The long-term clinical follow-up and natural history of men with adult-onset idiopathic hypogonadotropic hypogonadism. J Clin Endocrinol Metab. 2010; 95(9):4235–43. [PubMed: 20591981]
5. Kallmann FJ, Schoenfeld W, Barrera S. The genetic aspects of primary eunuchoidism. Am J Ment Defic. 1944; 48:203–36.
6. Quinton R, Duke VM, Kirk JM, Matfin G, de Zoysa PA, et al. Idiopathic gonadotrophin deficiency: genetic questions addressed through phenotypic characterization. Clin Endocrinol. 2001; 55(2):163–74.
7. Balasubramanian R, Dwyer A, Seminara SB, Pitteloud N, Kaiser UB, Crowley WF Jr, et al. Human GnRH deficiency: a unique disease model to unravel the ontogeny of GnRH neurons. Neuroendocrinology. 2010; 92(2):81–99. [PubMed: 20606386]

8. Crowley WF. The developmental biology of the GnRH neurons. Mol Cell Endocrinol. 2011; 346(1–2):1–3. [PubMed: 21741438]
9. Seminara SB, Hayes FJ, Crowley WF Jr. Gonadotropin-releasing hormone deficiency in the human (idiopathic hypogonadotropic hypogonadism and Kallmann's syndrome): pathophysiological and genetic considerations. Endocr Rev. 1998; 19(5):521–39. [PubMed: 9793755]
10. Sykiotis GP, Plummer L, Hughes VA, Au M, Durrani S, Nayak-Young S, et al. Oligogenic basis of isolated gonadotropin-releasing hormone deficiency. Proc Natl Acad Sci U S A. 2010; 107(34): 15140–4. [PubMed: 20696889]
11. Quaynor SD, Kim HG, Cappello EM, Williams T, Chorich LP, Bick DP, et al. The prevalence of digenic mutations in patients with normosmic hypogonadotropic hypogonadism and Kallmann syndrome. Fertil Steril. 2011; 96(6):1424–30 [e6]. [PubMed: 22035731]
12. Pitteloud N, Quinton R, Pearce S, Raivio T, Acierno J, Dwyer A, et al. Digenic mutations account for variable phenotypes in idiopathic hypogonadotropic hypogonadism. J Clin Invest. 2007; 117(2):457–63. [PubMed: 17235395]
13. Stamou MI, Cox KH, Crowley WF Jr. Discovering genes essential to the hypothalamic regulation of human reproduction using a human disease model: adjusting to life in the "-omics" era. Endocr Rev. 2015; 2016(1):4–22.
14. Perry JR, Day F, Elks CE, Sulem P, Thompson DJ, Ferreira T, et al. Parent-of-origin-specific allelic associations among 106 genomic loci for age at menarche. Nature. 2014; 514(7520):92–7. [PubMed: 25231870]
15. Perry JR, Corre T, Esko T, Chasman DI, Fischer K, Franceschini N, et al. A genome-wide association study of early menopause and the combined impact of identified variants. Hum Mol Genet. 2013; 22(7):1465–72. [PubMed: 23307926]
16. Rahmani M, Earp MA, Ramezani Tehrani F, Ataee M, Wu J, et al. Shared genetic factors for age at natural menopause in Iranian and European women. Hum Reprod. 2013; 28(7):1987–94. [PubMed: 23592221]
17. Chen CT, Liu CT, Chen GK, Andrews JS, Arnold AM, Dreyfus J, et al. Meta-analysis of loci associated with age at natural menopause in African-American women. Hum Mol Genet. 2014; 23(12):3327–42. [PubMed: 24493794]
18. Wray S, Grant P, Gainer H. Evidence that cells expressing luteinizing hormone-releasing hormone mRNA in the mouse are derived from progenitor cells in the olfactory placode. Proc Natl Acad Sci U S A. 1989; 86(20):8132–6. [PubMed: 2682637]
19. Schwanzel-Fukuda M, Bick D, Pfaff DW. Luteinizing hormone-releasing hormone (LHRH)-expressing cells do not migrate normally in an inherited hypogonadal (Kallmann) syndrome. Brain Res Mol Brain Res. 1989; 6(4):311–26. [PubMed: 2687610]
20. Barnett SC. Olfactory ensheathing cells: unique glial cell types? J Neurotrauma. 2004; 21(4):375–82. [PubMed: 15115587]
21. Couly GF, Le Douarin NM. Mapping of the early neural primordium in quail-chick chimeras. I. Developmental relationships between placodes, facial ectoderm, and prosencephalon. Dev Biol. 1985; 110(2):422–39. [PubMed: 4018406]
22. Murdoch B, DelConte C, Garcia-Castro MI. Embryonic Pax7-expressing progenitors contribute multiple cell types to the postnatal olfactory epithelium. J Neurosci. 2010; 30(28):9523–32. [PubMed: 20631180]
23. Whitlock KE. Development of the nervus terminalis: origin and migration. Microsc Res Tech. 2004; 65(1–2):2–12. [PubMed: 15570589]
24. Whitlock KE. A new model for olfactory placode development. Brain Behav Evol. 2004; 64(3): 126–40. [PubMed: 15353905]
25. Schlosser G. Making senses development of vertebrate cranial placodes. Int Rev Cell Mol Biol. 2010; 283:129–234. [PubMed: 20801420]
26. Nagoshi N, Shibata S, Kubota Y, Nakamura M, Nagai Y, Satoh E, et al. Ontogeny and multipotency of neural crest-derived stem cells in mouse bone marrow, dorsal root ganglia, and whisker pad. Cell Stem Cell. 2008; 2(4):392–403. [PubMed: 18397758]

27. Forni PE, Taylor-Burds C, Melvin VS, Williams T, Wray S, et al. Neural crest and ectodermal cells intermix in the nasal placode to give rise to GnRH-1 neurons, sensory neurons, and olfactory ensheathing cells. J Neurosci. 2011; 31(18):6915–27. [PubMed: 21543621]
28. Andersson AM, Toppari J, Haavisto AM, Petersen JH, Simell T, Simell O, et al. Longitudinal reproductive hormone profiles in infants: peak of inhibin B levels in infant boys exceeds levels in adult men. J Clin Endocrinol Metab. 1998; 83(2):675–81. [PubMed: 9467591]
29. Balasubramanian R, Crowley WF Jr. Isolated GnRH deficiency: a disease model serving as a unique prism into the systems biology of the GnRH neuronal network. Mol Cell Endocrinol. 2011; 346(1–2):4–12. [PubMed: 21782888]
30. Franco B, Guioli S, Pragliola A, Incerti B, Bardoni B, Tonlorenzi R, et al. A gene deleted in Kallmann's syndrome shares homology with neural cell adhesion and axonal path-finding molecules. Nature. 1991; 353(6344):529–36. [PubMed: 1922361]
31. Hardelin JP, Julliard AK, Moniot B, Soussi-Yanicostas N, Verney C, Schwanzel-Fukuda M, et al. Anosmin-1 is a regionally restricted component of basement membranes and interstitial matrices during organogenesis: implications for the developmental anomalies of X chromosome-linked Kallmann syndrome. Dev Dyn. 1999; 215(1):26–44. [PubMed: 10340754]
32. Kirk JM, Grant DB, Besser GM, Shalet S, Quinton R, Smith CS, et al. Unilateral renal aplasia in X-linked Kallmann's syndrome. Clin Genet. 1994; 46(3):260–2. [PubMed: 7820942]
33. Georgopoulos NA, Koika V, Galli-Tsinopoulou A, Spiliotis BE, Adonakis G, Keramida MK, et al. Renal dysgenesis and KAL1 gene defects in patients with sporadic Kallmann syndrome. Fertil Steril. 2007; 88(5):1311–7. [PubMed: 17603054]
34. Costa-Barbosa FA, Balasubramanian R, Keefe KW, Shaw ND, Al-Tassan N, Plummer L, et al. Prioritizing genetic testing in patients with Kallmann syndrome using clinical phenotypes. J Clin Endocrinol Metab. 2013; 98(5):E943–3. [PubMed: 23533228]
35. Vermeulen S, Messiaen L, Scheir P, De Bie S, Speleman F, De Paepe A. Kallmann syndrome in a patient with congenital spherocytosis and an interstitial 8p11.2 deletion. Am J Med Genet. 2002; 108(4):315–8. [PubMed: 11920837]
36. Dodé C, Levilliers J, Dupont JM, De Paepe A, Le Dû N, Soussi-Yanicostas N, et al. Loss-of-function mutations in FGFR1 cause autosomal dominant Kallmann syndrome. Nat Genet. 2003; 33(4):463–5. [PubMed: 12627230]
37. Pitteloud N, Meysing A, Quinton R, Acierno JS Jr, Dwyer AA, Plummer L, et al. Mutations in fibroblast growth factor receptor 1 cause Kallmann syndrome with a wide spectrum of reproductive phenotypes. Mol Cell Endocrinol. 2006; 254–255:60–9.
38. Falardeau J, Chung WC, Beenken A, Raivio T, Plummer L, Sidis Y, et al. Decreased FGF8 signaling causes deficiency of gonadotropin-releasing hormone in humans and mice. J Clin Invest. 2008; 118(8):2822–31. [PubMed: 18596921]
39. Miraoui H, Dwyer AA, Sykiotis GP, Plummer L, Chung W, Feng B, et al. Mutations in FGF17, IL17RD, DUSP6, SPRY4, and FLRT3 are identified in individuals with congenital hypogonadotropic hypogonadism. Am J Hum Genet. 2013; 92(5):725–43. [PubMed: 23643382]
40. Pitteloud N, Zhang C, Pignatelli D, Li JD, Raivio T, Cole LW, et al. Loss-of-function mutation in the prokineticin 2 gene causes Kallmann syndrome and normosmic idiopathic hypogonadotropic hypogonadism. Proc Natl Acad Sci U S A. 2007; 104(44):17447–52. [PubMed: 17959774]
41. Cole LW, Sidis Y, Zhang C, Quinton R, Plummer L, Pignatelli D, et al. Mutations in prokineticin 2 and prokineticin receptor 2 genes in human gonadotrophin-releasing hormone deficiency: molecular genetics and clinical spectrum. J Clin Endocrinol Metab. 2008; 93(9):3551–9. [PubMed: 18559922]
42. Cheng MY, Leslie FM, Zhou QY. Expression of prokineticins and their receptors in the adult mouse brain. J Comp Neurol. 2006; 498(6):796–809. [PubMed: 16927269]
43. Ng KL, Li JD, Cheng MY, Leslie FM, Lee AG, Zhou QY, et al. Dependence of olfactory bulb neurogenesis on prokineticin 2 signaling. Science. 2005; 308(5730):1923–7. [PubMed: 15976302]
44. Prosser HM, Bradley A, Caldwell MA. Olfactory bulb hypoplasia in Prokr2 null mice stems from defective neuronal progenitor migration and differentiation. Eur J Neurosci. 2007; 26(12):3339–44. [PubMed: 18052978]

45. Kramer PR, Wray S. Novel gene expressed in nasal region influences outgrowth of olfactory axons and migration of luteinizing hormone-releasing hormone (LHRH) neurons. Genes Dev. 2000; 14(14):1824–34. [PubMed: 10898796]
46. Miura K, Acierno JS Jr, Seminara SB. Characterization of the human nasal embryonic LHRH factor gene, NELF, and a mutation screening among 65 patients with idiopathic hypogonadotropic hypogonadism (IHH). J Hum Genet. 2004; 49(5):265–8. [PubMed: 15362570]
47. Kim HG, Ahn JW, Kurth I, Ullmann R, Kim HT, Kulharya A, et al. WDR11, a WD protein that interacts with transcription factor EMX1, is mutated in idiopathic hypogonadotropic hypogonadism and Kallmann syndrome. Am J Hum Genet. 2010; 87(4):465–79. [PubMed: 20887964]
48. Tornberg J, Sykiotis GP, Keefe K, Plummer L, Hoang X, Hall JE, et al. Heparan sulfate 6-O-sulfotransferase 1, a gene involved in extracellular sugar modifications, is mutated in patients with idiopathic hypogonadotrophic hypogonadism. Proc Natl Acad Sci U S A. 2011; 108(28):11524–9. [PubMed: 21700882]
49. Young J, Metay C, Bouligand J, Tou B, Francou B, Maione L, et al. SEMA3A deletion in a family with Kallmann syndrome validates the role of semaphorin 3A in human puberty and olfactory system development. Hum Reprod. 2012; 27(5):1460–5. [PubMed: 22416012]
50. Hanchate NK, Giacobini P, Lhuillier P, Parkash J, Espy C, Fouveaut C, et al. SEMA3A, a gene involved in axonal pathfinding, is mutated in patients with Kallmann syndrome. PLoS Genet. 2012; 8(8):e1002896. [PubMed: 22927827]
51. Cariboni A, André V, Chauvet S, Cassatella D, Davidson K, Caramello A, et al. Dysfunctional SEMA3E signaling underlies gonadotropin-releasing hormone neuron deficiency in Kallmann syndrome. J Clin Invest. 2015; 125(6):2413–28. [PubMed: 25985275]
52. Kim HG, Kurth I, Lan F, Meliciani I, Wenzel W, Eom SH, et al. Mutations in CHD7, encoding a chromatin-remodeling protein, cause idiopathic hypogonadotropic hypogonadism and Kallmann syndrome. Am J Hum Genet. 2008; 83(4):511–9. [PubMed: 18834967]
53. Jongmans MC, van Ravenswaaij-Arts CM, Pitteloud N, Ogata T, Sato N, Claahsen-van der Grinten HL, et al. CHD7 mutations in patients initially diagnosed with Kallmann syndrome–the clinical overlap with CHARGE syndrome. Clin Genet. 2009; 75(1):65–71. [PubMed: 19021638]
54. Balasubramanian R, Choi JH, Francescatto L, Willer J, Horton ER, Asimacopoulos EP, et al. Functionally compromised CHD7 alleles in patients with isolated GnRH deficiency. Proc Natl Acad Sci U S A. 2014; 111(50):17953–8. [PubMed: 25472840]
55. Marcos S, Sarfati J, Leroy C, Fouveaut C, Parent P, Metz C, et al. The prevalence of CHD7 missense versus truncating mutations is higher in patients with Kallmann syndrome than in typical CHARGE patients. J Clin Endocrinol Metab. 2014; 99(10):E2138–3. [PubMed: 25077900]
56. Fujita K, Ogawa R, Ito K. CHD7, Oct3/4, Sox2, and Nanog control FoxD3 expression during mouse neural crest-derived stem cell formation. FEBS J. 2016; 283(20):3791–806. [PubMed: 27579714]
57. Bajpai R, Chen DA, Rada-Iglesias A, Zhang J, Xiong Y, Helms J, et al. CHD7 cooperates with PBAF to control multipotent neural crest formation. Nature. 2010; 463(7283):958–62. [PubMed: 20130577]
58. Schulz Y, Wehner P, Opitz L, Salinas-Riester G, Bongers EM, van Ravenswaaij-Arts CM, et al. CHD7, the gene mutated in CHARGE syndrome, regulates genes involved in neural crest cell guidance. Hum Genet. 2014; 133(8):997–1009. [PubMed: 24728844]
59. Pingault V, Bodereau V, Baral V, Marcos S, Watanabe Y, Chaoui A, et al. Loss-of-function mutations in SOX10 cause Kallmann syndrome with deafness. Am J Hum Genet. 2013; 92(5): 707–24. [PubMed: 23643381]
60. Chew S, Balasubramanian R, Chan WM, Kang PB, Andrews C, Webb BD, et al. A novel syndrome caused by the E410K amino acid substitution in the neuronal beta-tubulin isotype 3. Brain. 2013; 136(Pt 2):522–35. [PubMed: 23378218]
61. Bondurand N, Pingault V, Goerich DE, Lemort N, Sock E, Le Caignec C, et al. Interaction among SOX10, PAX3 and MITF, three genes altered in Waardenburg syndrome. Hum Mol Genet. 2000; 9(13):1907–17. [PubMed: 10942418]

62. Margolin DH, Kousi M, Chan YM, Lim ET, Schmahmann JD, Hadjivassiliou M, et al. Ataxia, dementia, and hypogonadotropism caused by disordered ubiquitination. N Engl J Med. 2013; 368(21):1992–2003. [PubMed: 23656588]

63. Topaloglu AK, Lomniczi A, Kretzschmar D, Dissen GA, Kotan LD, McArdle CA, et al. Loss-of-function mutations in PNPLA6 encoding neuropathy target esterase underlie pubertal failure and neurological deficits in Gordon Holmes syndrome. J Clin Endocrinol Metab. 2014; 99(10):E2067–5. [PubMed: 25033069]

64. Bernard G, Chouery E, Putorti ML, Tétreault M, Takanohashi A, Carosso G, et al. Mutations of POLR3A encoding a catalytic subunit of RNA polymerase Pol III cause a recessive hypomyelinating leukodystrophy. Am J Hum Genet. 2012; 89(3):415–23.

65. Tétreault M, Choquet K, Orcesi S, Tonduti D, Balottin U, Teichmann M, et al. Recessive mutations in POLR3B, encoding the second largest subunit of Pol III, cause a rare hypomyelinating leukodystrophy. Am J Hum Genet. 2011; 89(5):652–5. [PubMed: 22036172]

66. Kotan LD, Hutchins BI, Ozkan Y, Demirel F, Stoner H, Cheng PJ, et al. Mutations in FEZF1 cause Kallmann syndrome. Am J Hum Genet. 2014; 95(3):326–31. [PubMed: 25192046]

67. Tata B, Huijbregts L, Jacquier S, Csaba Z, Genin E, Meyer V, et al. Haploinsufficiency of Dmxl2, encoding a synaptic protein, causes infertility associated with a loss of GnRH neurons in mouse. PLoS Biol. 2014; 12(9):e1001952. [PubMed: 25248098]

68. Cerrato F, Shagoury J, Kralickova M, Dwyer A, Falardeau J, Ozata M, et al. Coding sequence analysis of GNRHR and GPR54 in patients with congenital and adult-onset forms of hypogonadotropic hypogonadism. Eur J Endocrinol. 2006; 155(Suppl. 1):S3–S10. [PubMed: 17074994]

69. Chan YM, de Guillebon A, Lang-Muritano M, Plummer L, Cerrato F, Tsiaras S, et al. GNRH1 mutations in patients with idiopathic hypogonadotropic hypogonadism. Proc Natl Acad Sci U S A. 2009; 106(28):11703–8. [PubMed: 19567835]

70. Seminara SB, Messager S, Chatzidaki EE, Thresher RR, Acierno JS Jr, Shagoury JK, et al. The GPR54 gene as a regulator of puberty. N Engl J Med. 2003; 349(17):1614–27. [PubMed: 14573733]

71. de Roux N, Genin E, Carel JC, Matsuda F, Chaussain JL, Milgrom E. Hypogonadotropic hypogonadism due to loss of function of the KiSS1-derived peptide receptor GPR54. Proc Natl Acad Sci U S A. 2003; 100(19):10972–6. [PubMed: 12944565]

72. Topaloglu AK, Tello JA, Kotan LD, Ozbek MN, Yilmaz MB, Erdogan S, et al. Inactivating KISS1 mutation and hypogonadotropic hypogonadism. N Engl J Med. 2012; 366(7):629–35. [PubMed: 22335740]

73. Irwig MS, Fraley GS, Smith JT, Acohido BV, Popa SM, Cunningham MJ, et al. Kisspeptin activation of gonadotropin releasing hormone neurons and regulation of KiSS-1 mRNA in the male rat. Neuroendocrinology. 2004; 80(4):264–72. [PubMed: 15665556]

74. Matsui H, Takatsu Y, Kumano S, Matsumoto H, Ohtaki T. Peripheral administration of metastin induces marked gonadotropin release and ovulation in the rat. Biochem Biophys Res Commun. 2004; 320(2):383–8. [PubMed: 15219839]

75. Lippincott MF, Chan YM, Delaney A, Rivera-Morales D, Butler JP, Seminara SB, et al. Kisspeptin responsiveness signals emergence of reproductive endocrine activity: implications for human puberty. J Clin Endocrinol Metab. 2016; 101(8):3061–9. [PubMed: 27214398]

76. Chan YM, Lippincott MF, Butler JP, Sidhoum VF, Li CX, Plummer L, et al. Exogenous kisspeptin administration as a probe of GnRH neuronal function in patients with idiopathic hypogonadotropic hypogonadism. J Clin Endocrinol Metab. 2014; 99(12):E2762–1. [PubMed: 25226293]

77. Chan YM. Effects of kisspeptin on hormone secretion in humans. Adv Exp Med Biol. 2013; 784:89–112. [PubMed: 23550003]

78. Chan YM, Butler JP, Sidhoum VF, Pinnell NE, Seminara SB, et al. Kisspeptin administration to women: a window into endogenous kisspeptin secretion and GnRH responsiveness across the menstrual cycle. J Clin Endocrinol Metab. 2012; 97(8):E1458–7. [PubMed: 22577171]

79. Abbara A, Ratnasabapathy R, Jayasena CN, Dhillo WS, et al. The effects of kisspeptin on gonadotropin release in non-human mammals. Adv Exp Med Biol. 2013; 784:63–87. [PubMed: 23550002]

80. Jayasena CN, Abbara A, Comninos AN, Nijher GM, Christopoulos G, Narayanaswamy S, et al. Kisspeptin-54 triggers egg maturation in women undergoing in vitro fertilization. J Clin Invest. 2014; 124(8):3667–77. [PubMed: 25036713]
81. Navarro VM, Castellano JM, Fernández-Fernández R, Barreiro ML, Roa J, Sanchez-Criado JE, et al. Developmental and hormonally regulated messenger ribonucleic acid expression of KiSS-1 and its putative receptor, GPR54, in rat hypothalamus and potent luteinizing hormone-releasing activity of KiSS-1 peptide. Endocrinology. 2004; 145(10):4565–74. [PubMed: 15242985]
82. Gottsch ML, Cunningham MJ, Smith JT, Popa SM, Acohido BV, Crowley WF, et al. A role for kisspeptins in the regulation of gonadotropin secretion in the mouse. Endocrinology. 2004; 145(9): 4073–7. [PubMed: 15217982]
83. Herbison AE, de Tassigny XD, Doran J, Colledge WH. Distribution and postnatal development of Gpr54 gene expression in mouse brain and gonadotropin-releasing hormone neurons. Endocrinology. 2010; 151(1):312–21. [PubMed: 19966188]
84. Clarkson J, d'Anglemont de Tassigny X, Colledge WH, Caraty A, Herbison AE, et al. Distribution of kisspeptin neurones in the adult female mouse brain. J Neuroendocrinol. 2009; 21(8):673–82. [PubMed: 19515163]
85. Topaloglu AK, Reimann F, Guclu M, Yalin AS, Kotan LD, Porter KM, et al. TAC3 and TACR3 mutations in familial hypogonadotropic hypogonadism reveal a key role for Neurokinin B in the central control of reproduction. Nat Genet. 2009; 41(3):354–8. [PubMed: 19079066]
86. Gianetti E, Tusset C, Noel SD, Au MG, Dwyer AA, Hughes VA, et al. TAC3/TACR3 mutations reveal preferential activation of gonadotropin-releasing hormone release by neurokinin B in neonatal life followed by reversal in adulthood. J Clin Endocrinol Metab. 2010; 95(6):2857–67. [PubMed: 20332248]
87. Navarro VM, Gottsch ML, Chavkin C, Okamura H, Clifton DK, Steiner RA, et al. Regulation of gonadotropin-releasing hormone secretion by kisspeptin/dynorphin/neurokinin B neurons in the arcuate nucleus of the mouse. J Neurosci. 2009; 29(38):11859–66. [PubMed: 19776272]
88. Goodman RL, Lehman MN, Smith JT, Coolen LM, de Oliveira CV, Jafarzadehshirazi MR, et al. Kisspeptin neurons in the arcuate nucleus of the ewe express both dynorphin A and neurokinin B. Endocrinology. 2007; 148(12):5752–60. [PubMed: 17823266]
89. Wakabayashi Y, Nakada T, Murata K, Ohkura S, Mogi K, Navarro VM, et al. Neurokinin B and dynorphin A in kisspeptin neurons of the arcuate nucleus participate in generation of periodic oscillation of neural activity driving pulsatile gonadotropin-releasing hormone secretion in the goat. J Neurosci. 2010; 30(8):3124–32. [PubMed: 20181609]
90. Seminara SB, Achermann JC, Genel M, Jameson JL, Crowley WF Jr, et al. X-linked adrenal hypoplasia congenita: a mutation in DAX1 expands the phenotypic spectrum in males and females. J Clin Endocrinol Metab. 1999; 84(12):4501–9. [PubMed: 10599709]
91. Strobel A, Issad T, Camoin L, Ozata M, Strosberg AD. A leptin missense mutation associated with hypogonadism and morbid obesity. Nat Genet. 1998; 18(3):213–5. [PubMed: 9500540]
92. Farooqi IS, Wangensteen T, Collins S, Kimber W, Matarese G, Keogh JM, et al. Clinical and molecular genetic spectrum of congenital deficiency of the leptin receptor. N Engl J Med. 2007; 356(3):237–47. [PubMed: 17229951]
93. Farooqi IS, Jebb SA, Langmack G, Lawrence E, Cheetham CH, Prentice AM, et al. Effects of recombinant leptin therapy in a child with congenital leptin deficiency. N Engl J Med. 1999; 341(12):879–84. [PubMed: 10486419]
94. Clément K, Vaisse C, Lahlou N, Cabrol S, Pelloux V, Cassuto D, et al. A mutation in the human leptin receptor gene causes obesity and pituitary dysfunction. Nature. 1998; 392(6674):398–401. [PubMed: 9537324]
95. Jackson RS, Creemers JW, Ohagi S, Raffin-Sanson ML, Sanders L, Montague CT, et al. Obesity and impaired prohormone processing associated with mutations in the human prohormone convertase 1 gene. Nat Genet. 1997; 16(3):303–6. [PubMed: 9207799]
96. Ahima RS, Prabakaran D, Mantzoros C, Qu D, Lowell B, Maratos-Flier E, et al. Role of leptin in the neuroendocrine response to fasting. Nature. 1996; 382(6588):250–2. [PubMed: 8717038]
97. Ahima RS, Dushay J, Flier SN, Prabakaran D, Flier JS. Leptin accelerates the onset of puberty in normal female mice. J Clin Invest. 1997; 99(3):391–5. [PubMed: 9022071]

98. Welt CK, Chan JL, Bullen J, Murphy R, Smith P, DePaoli AM, et al. Recombinant human leptin in women with hypothalamic amenorrhea. N Engl J Med. 2004; 351(10):987–97. [PubMed: 15342807]

99. Cravo RM, Margatho LO, Osborne-Lawrence S, Donato J Jr, Atkin S, Bookout AL, et al. Characterization of Kiss1 neurons using transgenic mouse models. Neuroscience. 2011; 173:37–56. [PubMed: 21093546]

100. Hausman GJ, Barb CR, Lents CA. Leptin and reproductive function. Biochimie. 2012; 94(10): 2075–81. [PubMed: 22980196]

101. Donato J Jr, Cravo RM, Frazão R, Elias CF. Hypothalamic sites of leptin action linking metabolism and reproduction. Neuroendocrinology. 2011; 93(1):9–18. [PubMed: 21099209]

102. Marshall WA, Tanner JM. Variations in the pattern of pubertal changes in boys. Arch Dis Child. 1970; 45(239):13–23. [PubMed: 5440182]

103. Marshall WA, Tanner JM. Variations in pattern of pubertal changes in girls. Arch Dis Child. 1969; 44(235):291–303. [PubMed: 5785179]

104. Mao JF, Xu HL, Duan J, Chen RR, Li L, Li B, et al. Reversal of idiopathic hypogonadotropic hypogonadism. N Engl J Med. 2007; 357(9):863–73. [PubMed: 17761590]

105. Lunetta KL, Day FR, Sulem P, Ruth KS, Tung JY, Hinds DA, et al. Rare coding variants and X-linked loci associated with age at menarche. Nat Commun. 2015; 6:7756. [PubMed: 26239645]

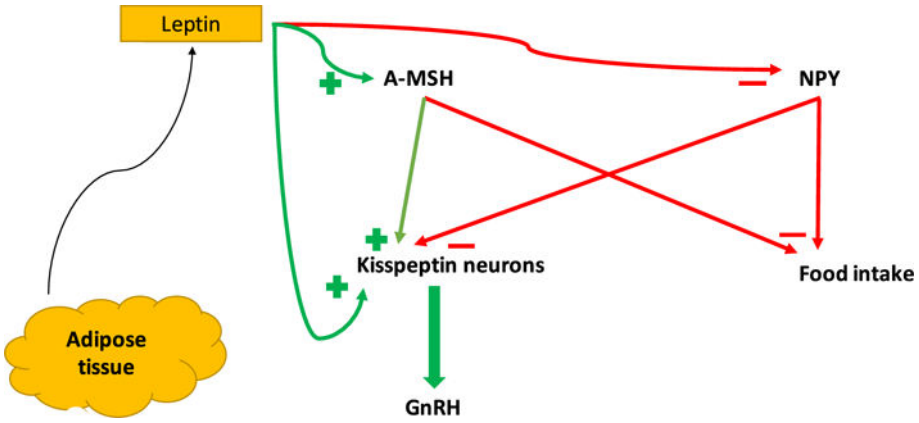

Fig. 1.

Effects of Leptin on the GnRH secretion and action. Leptin regulates the HPG axis by acting at the level of hypothalamus in three different ways, as shown above, including: (i) suppression of NPY (neuropeptide-Y) (ii) stimulation of a-MSH (melanocyte stimulating hormone) and (iii) direct interaction with subpopulations of kisspeptin neurons to further increase the stimulatory drive on GnRH release. Stimulatory effect is shown in green and inhibitory effect in red. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 1

Genes associated with Kallmann Syndrome and normosmic idiopathic hypogonadotropic hypogonadism and their characteristics.

Gene	Description	Chromosome	Function	Phenotype
KAL1	Kallmann 1	chrXp22.31	Neurodevelopmental	KS
NSMF	NMDA receptor synaptonuclear signaling and neuronal migration factor	chr9q34.3	Neurodevelopmental	KS and nIHH
FGFR1	Fibroblast growth factor receptor 1	chr8p11.23	Neurodevelopmental	KS and nIHH
FGF8	Fibroblast growth factor 8	chr10q24.32	Neurodevelopmental	KS, nIHH and AHH
FGF17	Fibroblast growth factor 17	chr8p21.3	Neurodevelopmental	KS and nIHH
IL17RD	Interleukin 17 receptor D	chr3p14.3	Neurodevelopmental	KS
DUSP6	Dual specificity phosphate 6	chr12q21.33	Neurodevelopmental	KS
SPRY4	Sprouty drosophila homolog of 4	chr5q31.3	Neurodevelopmental	KS and nIHH
GLCE	Glucuronic acid epierase	chr15q23	Neurodevelopmental	KS and nIHH
FLRT3	Fibronectin like domain containing leucine rich transmembrane protein 3	chr20p12.1	Neurodevelopmental	KS and nIHH
PROK2	Prokineticin 2	chr3p13	Neurodevelopmental	KS and nIHH
PROKR2	Prokineticin receptor 2	chr20p12.3	Neurodevelopmental	KS, nIHH and AHH
HS6ST1	Heparin sulfate 6 O sulfutransferase	chr2q14.3	Neurodevelopmental	KS and nIHH
CHD7	Chromodomain helicase DNA binding protein 7	chr8q12.2	Neurodevelopmental	KS and nIHH
WDR11	WD Repeat-Containing protein 11	crh10q26.12	Neurodevelopmental	KS and nIHH
SEMA3A	Semaphorin 3A	chr7q21.11	Neurodevelopmental	KS
SEMA3E	Semapthorin 3E	chr7q21.11	Neurodevelopmental	KS and nIHH
TUBB3	Tubulin beta 3	chr16q24.3	Neurodevelopmental	KS
SOX10	SRY box 10	chr22q13.1	Neurodevelopmental	KS
OTUD4	OUT domain containing protein 4	chr4q31.21	Neurodevelopmental	nIHH and ataxia
FEZF1	fez family zinc finger protein 1	chr7q31.32	Neurodevelopmental	KS
RNF216	Ring finger protein 216	chr7p22.1	Neurodevelopmental	nIHH and ataxia
POLR3A	Polymerase III RNA subunit A	chr10q22.3	Neurodevelopmental	nIHH and ataxia
POLR3B	Polymerase III RNA subunit B	chr12q23.3	Neurodevelopmental	nIHH and ataxia
PNPLA6	Patatin-like phospholipase domain-containing protein 6	chr19p13.2	Neurodevelopmental	nIHH and ataxia
STUB1	Stip1 homologous and U box containing protein 1	chr16p13.3	Neurodevelopmental	nIHH and ataxia
DMXL2	DMX like 2	chr15q21.2	Neuroendocrine	nIHH and polyendcrin3-polyneuropahty syndrome
GNRH1	GnRH 1	chr8p21.2	Neuroendocrine	nIHH
GNRHR	GnRH Receptor	chr4q13.2	Neuroendocrine	nIHH and AHH
KISS1	Kisspeptin 1	chr1q32.1	Neuroendocrine	nIHH
KISS1R	Kisspeptin 1 receptor	chr19p13.3	Neuroendocrine	nIHH
TAC3	Tachykinin 3	chr12q13.3	Neuroendocrine	nIHH
TACR3	Tachykinin receptor 3	chr4q24	Neuroendocrine	nIHH
LEP	Leptin	chr7q32.1	Neuroendocrine	nIHH and obesity
LEPR	Leptin receptor	chr1p31.3	Neuroendocrine	nIHH and obesity
NR0B1	Nuclear receptor subfamily 0, group B, member 1	chrXp21.2	Neuroendocrine	nIHH

Known IGD genes and their characteristics including their description, chromosomal location, function and phenotype that are associated with. KS: Kallmann syndrome, nIHH: normosmic hypogonadotropic hypogonadism, AHH: adult-onset hypogonadotropic hypogonadism.