Genetics of hypogonadotropic hypogonadism

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Abstract: Male congenital hypogonadotropic hypogonadism (CHH) is a heterogenous group of genetic disorders that cause impairment in the production or action of gonadotropin releasing hormone (GnRH). These defects result in dysfunction of the hypothalamic-pituitary-gonadal hormone axis, leading to low testosterone levels and impaired fertility. Genetic testing techniques have expanded our knowledge of the underlying mechanisms contributing to CHH including over 30 genes to date implicated in the development of CHH. In some cases, non-reproductive signs or symptoms can give clues as to the putative genetic etiology, but many cases remain undiagnosed with less than 50% identified with a specific gene defect. This leads to many patients labelled as "idiopathic hypogonadotropic hypogonadism". Medical and family history as well as physical exam and laboratory features can aid in the identification of hypogonadotropic hypogonadism (HH) that is associated with specific medical syndromes or associated with other pituitary hormonal deficiencies. Genetic testing strategies are moving away from the classic practice of testing for only a few of the most commonly affected genes and instead utilizing next generation sequencing techniques that allow testing of numerous potential gene targets simultaneously. Treatment of CHH is dependent on the individual's desire to preserve fertility and commonly include human chorionic gonadotropin (hCG) and recombinant follicle stimulating hormone (rFSH) to stimulate testosterone production and spermatogenesis. In situations where fertility is not desired, testosterone replacement therapies are widely offered in order to maintain virilization and sexual function.

Keywords: Isolated hypogonadotropic hypogonadism; hypogonadism; hypopituitarism; congenital; infertility; male

Background

Estimates on prevalence of male hypogonadism vary greatly depending on the definition used to define low testosterone levels (1). Cases of hypogonadism can be broadly divided into primary (hypergonadotropic) hypogonadism, defined by low testosterone levels and reduced or absent sperm concentrations in the setting of elevated gonadotropin levels, and secondary (hypogonadotropic) hypogonadism (HH), defined by low testosterone levels and reduced or absent sperm concentrations in the setting of low or "inappropriately normal" gonadotropin levels. Each hypogonadal subtype can be further classified into acquired or congenital causes. While potentially one of the most treatable forms of male infertility, HH is a rare condition, representing <1% of cases seen in fertility clinics (2).

Congenital hypogonadotropic hypogonadism (CHH) is a heterogeneous group of inherited gene defects that

Table 1 Associated phenotypic features of select gene mutations [adapted from references (4,7)]

Affected gene	Associated features
ANOS1 (KAL1)	Cryptorchidism, small testes, may have unilateral renal agenesis, synkinesia (8)
SOX10	Waardenburg Syndrome: sensorineural deafness, skin, hair and iris pigmentation abnormalities, Hirschsprung's disease (9)
IL17RD	Hearing loss (10)
TAC3 and TAC3R	Microphallus and cryptorchidism, potential reversibility of hypogonadism in adulthood (11)
FGFR1	Cleft lip or palate, dental agenesis, bimanual synkinesis, iris coloboma, possible agenesis of corpus callosum, unilateral hearing loss, digital malformations (brachydactyly, syndactyly) (12)
FGF8	Hearing loss, high arched palate, cleft lip/palate, severe osteoporosis, camplodactyly, digit hyperlaxity, microphallus, cryptorchidism, flat nasal bridge, hypertelorism (13)
CHD7	CHARGE syndrome: coloboma of eye, heart defects, choanal atresia, retarded growth and development, genital hypoplasia, dysmorphic ears and/or hypoplasia or aplasia of semicircular canals and deafness (14)

ultimately result in HH. CHH has also been labelled in the literature as idiopathic hypogonadotropic hypogonadism, isolated hypogonadotropic hypogonadism or isolated gonadotropin-releasing hormone (GnRH) deficiency (3). For the purposes of this review, "CHH" will be used to describe HH due to diagnosed gene defects, while "isolated HH" will be reserved for cases in which a gene defect or other underlying etiology has not been identified.

CHH is recognized as having a male predominance, with estimates of male-to-female ratios ranging from 3–5 to 1 (4). Perhaps the most commonly recognized form of CHH is Kallmann syndrome, classically manifested by HH with lack of sense of smell (anosmia) or reduced sense of smell (hyposmia). Since the original description of Kallmann syndrome in 1944 (5), it has been recognized that there are a significant number of people with CHH who have a normal sense of smell. A Finnish study characterized the prevalence of Kallmann syndrome as representing 1 in 30,000 males and 1 in 125,000 females (6).

Although CHH is often grouped as one condition, it represents tremendous phenotypic and genotypic heterogeneity (*Table 1*). Patients can present with several non-reproductive signs and symptoms with over 30 genes implicated in genetic studies (3). There is significant variability in the inheritance patterns of CHH, including autosomal dominant, autosomal recessive, X-linked, and oligogenic inheritance, with further variations as a result of differences in gene penetrance and expressivity (15,16).

It is interesting to note that despite the identification of nearly three dozen CHH-related genes, a genetic etiology is still not identified in over 50% of cases (3),

making CHH often a diagnosis of exclusion. Because of this, it is essential to rule out other possible causes of hypogonadotropic hypogonadism, such as pituitary tumors, infiltrative conditions such as hemochromatosis, other genetic syndromes, or functional hypogonadism caused by medications or other medical conditions before applying a label of CHH. In the adolescent patient, it is equally important to rule out the possibility of constitutional delay of growth and puberty (CDGP), which has been reported to be as high as 65% of males presenting with delayed pubertal onset (17).

Pathophysiology

Unlike other neuroendocrine cells, GnRH secreting neurons begin their development outside of the central nervous system in the medial part of the nasal epithelium, where they migrate to the forebrain starting in week 6 of embryonic development (18,19). From here, the cells extend towards their ultimate locations in the arcuate nucleus and preoptic area of the hypothalamus. By week 15 of embryonic development the cells extend their axons towards the median eminence, where they can interact with the hypothalamo-pituitary portal vessels (3). GnRH, once secreted, enters the hypothalamo-pituitary portal vessels and travels to GnRH receptors located on gonadotropic cells in the anterior pituitary gland, ultimately binding to these receptors and resulting in secretion of luteinizing hormone (LH) and follicle stimulating hormone (FSH), and subsequent stimulation of various gonadal functions including testosterone production and spermatogenesis (4).

Table 2 Subtypes of CHH [adapted from references (4,7)]

Gene	Mode of inheritance	Anosmic/Normosmic	Gene product
ANOS1 (KAL1)	X-linked	Anosmic	Glycoprotein Anosmin-1 (26)
SEMA3A	Autosomal dominant with variable expressivity	Anosmic	Semaphorin 3A (27)
SOX10	Autosomal dominant with variable expressivity	Anosmic	Sex-determining region Y-Box 10 transcription factor (9)
IL17RD	Autosomal dominant with variable expressivity/autosomal recessive	Anosmic	Interleukin-17 receptor D (10)
FEZF1	Autosomal recessive	Anosmic	FEZ family zinc finger 1 (28)
FGFR1	Autosomal dominant with variable expressivity	Both	Fibroblast growth factor receptor 1 (12)
FGF8	Autosomal dominant with variable expressivity	Both	Fibroblast growth factor 8 (13)
PROK2 and PROKR2	Autosomal recessive/oligogenic	Both	Prokineticin 2 and its receptor (29,30)
CHD7	Autosomal dominant with variable expressivity	Both	Chromodomain helicase DNA-binding protein 7 (14)
NSMF	Oligogenic	Both	NMDA [N-methyl-D-aspartate] receptor synaptonuclear signaling and neuronal migration factor (NSMF) (31)
HS6ST1	Autosomal dominant/oligogenic	Both	Heparin sulfate 6-o-sulfotransferase 1 (32)
FGF17	Autosomal dominant/oligogenic	Both	Fibroblast Growth Factor 17 (10)
SPRY4	Autosomal dominant/oligogenic	Both	Sprouty homolog 4 (10)
DUSP6	Autosomal dominant/oligogenic	Both	MKP3-Mitogen-activated protein kinase (MAPK) phosphatase (10)
FLRT3	Indeterminate/oligogenic	Both	Fibronectin leucine rich transmembrane protein 3 (10,33)
WDR11	Indeterminate/oligogenic	Both	WD repeat-containing protein 11 (34)
AXL	Indeterminate/oligogenic	Both	AXL receptor tyrosine kinase (35)
GNRHR	Autosomal recessive	Normosmic	GnRH receptor (36)
GNRH1	Autosomal recessive	Normosmic	PreproGnRH (37)
KISS1R	Autosomal recessive	Normosmic	Kisspeptin receptor 1 (38)
KISS1	Autosomal recessive	Normosmic	Kisspeptin (39)
TAC3	Autosomal recessive	Normosmic	Neurokinin B (11,40)
TAC3R	Autosomal recessive	Normosmic	Neurokinin B receptor (11,40)

As olfactory neurons develop in close proximity to GnRH secreting neurons in the nasal placode and migrate along similar pathways, it can be understood how anosmia or hyposmia often occurs in conjunction with CHH (20,21). In many cases of Kallmann syndrome structural changes of the olfactory bulbs such as partial bulb development or complete aplasia can be visualized on brain MRI scans (22,23). It should be noted, however, that a normal MRI scan does not rule out a diagnosis of Kallmann syndrome, as normal olfactory bulbs can still be visualized in 20% of cases (24,25).

GnRH neuronal development is a complex process, with potential for defects at several stages of its course. As stated previously, nearly three dozen genes have been implicated in the development of CHH, with examples listed in *Table 2*. It has been proposed by Young et al. that defects that occur

during the development and migration of GnRH secreting neurons can ultimately lead to CHH via four broadly defined mechanisms:

(I) Defects in GnRH fate specification.
(II) Defects in GnRH neuron migration/olfactory neuron guidance.
(III) Abnormal neuroendocrine secretion/homeostasis.
(IV) Gonadotrope defects (3).

Clinical presentation

The presentation of individuals with CHH is dependent on the life stage at which the patient is assessed. Often the diagnosis is not made until adolescence, when lack of pubertal development may prompt further investigations. Neonatal boys with CHH may present earlier with micropenis and cryptorchidism due to a lack of GnRH stimulation (41). Signs and symptoms of CHH are more readily recognized during adolescence, though, as patients will demonstrate either partial or complete lack of secondary sexual characteristics, including absence of testicular and penile enlargement (42). Lack of pubertal progression is not diagnostic of CHH per se, as there are several other etiologies that must be considered (most notably CDGP). Affected individuals may also present later with concerns related to infertility, low libido, or erectile dysfunction (43).

Individuals with CHH can additionally present with non-reproductive symptoms and signs. Certain gene mutations known to cause isolated HH may predispose patients to other phenotypic changes that should prompt one to suspect a diagnosis of CHH (*Table 1*). Patients with classic Kallmann syndrome, for example, present with anosmia or hyposmia.

Distinguishing primary causes of isolated HH (i.e., CHH) from secondary or syndromic causes of isolated HH can be a challenge. It may require careful history taking, close review of family history, biochemical workup and imaging studies. Physical examination can be helpful to identify physical features that point towards a syndromic cause.

There are other conditions that affect multiple endocrine pathways in addition to HH. Individuals with obesity syndromes caused by mutations in *PCSK1*, *LEP*, and *LEPR* can develop HH (44-46). X-linked adrenal hypoplasia congenita caused by mutations in *NROB1* (*DAX1*) not only demonstrate adrenal failure in early childhood but may also cause HH in young adulthood (47). Combined pituitary hormone deficiency caused by *HESX1*, *LHX3*, *LXH4*, *POU1F1*, or *PROP1* can result in various degrees of hypopituitarism with HH prior to the development of other endocrinological disturbances (48).

Further, chromosomal differences or other rare gene defects can predispose individuals to HH as part of larger syndromic diagnosis. The more common syndromes are outlined in *Table 3* with their other associated features.

Practical aspects to genetic testing and counseling

Genetic testing for HH

In the past, the recommended testing strategies prioritized the order of genetic testing based on clinical presentation (e.g., presence or absence of anosmia), testing of mutations with a higher detection rate (e.g., *ANOS1*), or by inheritance pattern. Next-generation sequencing technology is now available and has allowed for multiple genes to be sequenced efficiently and simultaneously as comprehensive HH genetic panels. As such, these historic testing strategies may not be rational or cost-effective. If after careful history taking, review of family history, laboratory workup and imaging studies a diagnosis of isolated HH is suspected, genetic testing should be considered.

Genetic testing in the form of a next-generation sequencing panel of genes associated with isolated HH is recommended (multiple genes sequenced simultaneously) unless there is a known familial mutation, a clear inheritance pattern in the family, or a specific feature that points towards specific causal genes. This should include at a minimum the common genes associated with Kallmann and normosmic HH including *ANOS1*, *CHD7*, *FGFR1*, *GNRHR*, *IL17RD*, *PROKR2*, *SOX10*, and *TACR3*. There are multiple genetic testing companies that offer genetic panels for HH that include the common genes noted above but also all of the rarer genes found in *Tables 1* and *2*, with variable but comparable costs. The benefit of a larger HH panel to include rare genes is the potential to detect a less common cause or contributor to CHH, especially given the increasing number of CHH genes implicated in oligogenic disease. However, a possible downside of larger panels is the increased likelihood of detecting variants of unknown significance that may complicate genetic counselling of patients and families.

Any patient with developmental delay, and/or multiple congenital anomalies, and/or dysmorphic facial features should have chromosomal microarray testing to rule out copy

Table 3 Common syndromes causing hypogonadotropic hypogonadism with their associated features

Syndrome	Gene(s)	Associated features
Bardet-Biedl syndrome	20+ genes (highest yield: *BBS1*, *BBS10*, *BBS2*, *BBS9*, *MKKS*, *BBS12*, *MKS1*, *BBS4*, *BBS7*, *BBS7*, *TTC8*)	Obesity, intellectual disability, retinal abnormalities, polydactyly, renal abnormalities (49)
CHARGE syndrome	*CHD7*	Coloboma of eye, heart defects, choanal atresia, retarded growth and development, genital hypoplasia, dysmorphic ears and/or hypoplasia or aplasia of semicircular canals and deafness (14)
Gordon-Holmes syndrome	*OTUD4*, *PNPLA6*, *RNF216*, *STUB1*	Ataxia, dementia (4)
HFE-associated hereditary hemochromatosis	*HFE*	Liver dysfunction, diabetes, cardiomyopathy, arthritis (50)
Prader-Will syndrome	Loss of paternal 15q11.2	Obesity, developmental delay (51)
TUBB3 E410K syndrome	*TUBB3*	Congenital fibrosis of extraocular muscles, facial weakness, neuropathy, developmental delay (52)
Warburg Micro syndrome/ Martsolf syndrome	*RAB3GAP1*, *RAB3GAP2*, *RAB18*, *TBC1D20*	Congenital cataracts, microphthalmia, developmental delay, brain malformations (53)
Waardenburg syndrome	*SOX10*	Sensorineural deafness, skin, hair and iris pigmentation abnormalities, Hirschsprung's disease (9)
Xp21 deletion syndrome	Xp21 microdeletion encompassing *NROB1* (*DAX1*) and *DMD*	Primary adrenal insufficiency, Duchenne muscular dystrophy, developmental delay, seizures, acidosis, hypoglycemia (54)
Xp22.3 deletion syndrome	Xp22.3 microdeletion encompassing *ANOS1*	Kallmann syndrome, chondrodysplasia punctate, and ichthyosis (3)

number variations and a referral to a clinical geneticist for evaluation and testing for possible syndromic causes of HH.

Genetic counseling for HH

Genetic testing can not only confirm a diagnosis and underlying etiology of HH but can be useful for genetic counseling and prognosis. Patients found to have a pathogenic variant (mutation) or variant(s) of unknown significance on an HH genetics panel should be referred to a genetics centre for review and appropriate counseling. Once a mutation is found in a gene causing HH, the patient can be counseled appropriately with regards to risks to family members and future offspring.

ANOS1-related CHH is X-linked and will thus preferentially affect males. A male with a mutation in *ANOS1* has no chance of passing the condition on to male offspring, and his mother is eligible for carrier testing. All of his daughters will be obligate carriers for the condition and can potentially demonstrate features of hypogonadism, albeit less frequently. Autosomal conditions causing HH can affect males and females equally. In HH conditions that

are autosomal dominant, there is a 50% risk of transmission to their offspring. Depending on the mutation status in parents, there can be an up to 50% risk to siblings as well. Most dominant forms of HH demonstrate reduced penetrance and variable expressivity. For recessive HH conditions, the risk of transmission to offspring is very low unless there is consanguinity. As mentioned previously, genetic counselling for individuals who carry mutations in different CHH genes causing oligogenic HH can be more challenging (55).

Genetic testing can also aid in prognostication and for personalized management of patients. For example, *TAC3* and *TAC3R* mutations can cause CHH that may demonstrate reversibility in adulthood (11). Confirmation of a syndromic genetic diagnosis with potential features outside of the endocrine system (e.g., *CHD7*) may require additional screening and more complex medical management.

Management

Several treatment regimens exist for CHH with clinical

decision making guided by treatment goals, specifically related to fertility, and patient preference. Treatments available for induction or replacement of testicular function include GnRH, gonadotropins [human chorionic gonadotropin (hCG) and recombinant FSH (rFSH)], or testosterone replacement therapy (TRT). While TRT plays a role in inducing secondary sexual characteristics, administration of GnRH or gonadotropins are necessary to induce and support spermatogenesis (56). Given the challenges of administration and cost of GnRH and gonadotropins, peripubertal adolescent males with CHH are often initiated on TRT for puberty induction. Alternative treatment protocols must be considered once fertility is a concern. There are no standardized treatment regimens for CHH, therefore the recommendations below are largely based on observational studies and expert opinion.

Young men with CHH ready to start a family often have been on TRT for several years following induction of puberty. Fortunately, fertility can be restored in most CHH men with physiologic-replicating protocols utilizing GnRH or gonadotropins. GnRH, when given in a pulsatile manner, stimulates release of natural FSH and LH from the anterior pituitary. Delivered via a subcutaneous pump, pulses of 25 ng/kg are delivered every 90–120 min (3). FSH/LH ratio varies based on GnRH dose thus pulse dosage is adjusted for the ideal physiologic response (57). Not available in many countries and requiring a high level of expertise, GnRH is rarely used outside of research settings.

Bypassing the pituitary, the testes of CHH men can be directly stimulated with gonadotropins. FSH is available in recombinant form as an injectable. LH is not commercially available but hCG can be substituted due to its comparable chemical structure. While hCG monotherapy is sufficient to induce androgen production in CHH, it is less efficacious as a solitary puberty induction agent in CHH (58). Once fertility is a priority, CHH men should stop other androgen replacement protocols and initiate hCG subcutaneous injections of 500–1,500 IU three times weekly, adjusting dosage based on testosterone response (4). Side effects may include gynecomastia and erythrocytosis. Men with baseline severe testicular atrophy (volume <4 mL) or cryptorchidism should be counselled that hCG monotherapy has very low success rates and they will likely require a combination protocol (4,59).

In men with no sperm at 3–6 months or low chances of fertility restoration with hCG monotherapy, rFSH should be added, starting at 75–150 IU subcutaneously three times weekly and adjusting dosage based on serum FSH and sperm count. Despite possible dosage increases, it is apparent that testicular potential, namely volume and sperm production, may be limited in men with CHH. Most reports suggest men with smaller testicular volumes pre-treatment will have smaller volumes post-treatment (58), and though spermatogenesis can be induced in most men (64–95% in reported series), many will have oligospermia (3). A 2014 meta-analysis cited a mean sperm concentration of 5.9×10^6/mL following gonadotropin optimization (60). Promisingly, small series have suggested that the sperm concentration needed for conception among CHH men may be lower than World Health Organization reference values for fertile men (61). As gonadotropin treatment is continued, decreasing responsiveness to hCG may occur as a result of antibody induction (62).

FSH priming has received special attention in the literature. The rationale for this strategy stems from the fact that men with severe GnRH deficiency have a compromised Sertoli cell population which can be potentially optimized with rFSH before hCG. A single randomized controlled study of 13 young men demonstrated promising results with more patients in the treatment arm developing sperm in the ejaculate and trending towards higher sperm counts (63). All men had initial testicular volumes of <4 mL. Priming requires 2–4 months of initial rFSH, an intensive and expensive protocol. Further studies are needed to determine the potential benefits and ideal CHH population for FSH priming.

Regardless of the testicular-stimulating protocol selection, sperm cryopreservation should be considered when found, even if fertility is not an active priority. Any sperm retrieved and frozen can be used later with assisted reproductive technologies (ART). Likewise, if spermatogenesis has been restored but a CHH man and his partner are unable to conceive naturally, referral should be made to a reproductive endocrinologist for complete female partner evaluation and ART discussion. Once done with family planning, most men with CHH will transition back to TRT.

Testosterone replacement can be accomplished with a variety of formulations including topical gels, nasal gel, patches, short and long-acting injectables, and pellets. Classic side effects of TRT includes skin changes (e.g., acne), gynecomastia, edema, infertility, worsening of obstructive sleep apnea and erythrocytosis. Effects on prostate and cardiac health remain controversial topics and are beyond the scope of the present review but should be

discussed with patients. Topical options carry unique risk of transference to other individuals at home, a particular concern in CHH men who may have just started a family with the treatments noted above. Selection should be made based on patient preference, formulation-specific side effect profile, and availability.

Conclusions

Although CHH is a relatively uncommon cause of male infertility, it is one of the most treatable causes due to the availability and effectiveness of GnRH and gonadotropin replacement regimens. Ongoing advances in genetic testing and identification will hopefully lead to more men being diagnosed with a specific etiology thereby allowing targeted treatment options and informing requirements for genetic testing of family members and future offspring.

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