EXPERT CONSENSUS DOCUMENT

European Consensus Statement on congenital hypogonadotropic hypogonadism—pathogenesis, diagnosis and treatment

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Abstract | Congenital hypogonadotropic hypogonadism (CHH) is a rare disorder caused by the deficient production, secretion or action of gonadotropin-releasing hormone (GnRH), which is the master hormone regulating the reproductive axis. CHH is clinically and genetically heterogeneous, with >25 different causal genes identified to date. Clinically, the disorder is characterized by an absence of puberty and infertility. The association of CHH with a defective sense of smell (anosmia or hyposmia), which is found in ~50% of patients with CHH is termed Kallmann syndrome and results from incomplete embryonic migration of GnRH-synthesizing neurons. CHH can be challenging to diagnose, particularly when attempting to differentiate it from constitutional delay of puberty. A timely diagnosis and treatment to induce puberty can be beneficial for sexual, bone and metabolic health, and might help minimize some of the psychological effects of CHH. In most cases, fertility can be induced using specialized treatment regimens and several predictors of outcome have been identified. Patients typically require lifelong treatment, yet ~10–20% of patients exhibit a spontaneous recovery of reproductive function. This Consensus Statement summarizes approaches for the diagnosis and treatment of CHH and discusses important unanswered questions in the field.

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Introduction

Congenital hypogonadotropic hypogonadism (CHH) is caused by deficient production, secretion or action of gonadotropin-releasing hormone (GnRH), a key neuropeptide that orchestrates mammalian reproduction.[1] CHH is characterized by incomplete or absent puberty and infertility in the setting of isolated hypogonadotropic hypogonadism (that is, otherwise normal anterior pituitary function). CHH can present solely as congenital GnRH deficiency or be associated with other developmental anomalies such as cleft lip or palate, dental agenesis, ear anomalies, congenital hearing impairment, renal agenesis, bimanual synkinesis or skeletal anomalies. When associated with anosmia or hyposmia, CHH is termed Kallmann syndrome, which results from abnormal embryonic migration of GnRH neurons from their origin in the olfactory placode to the forebrain.[2,3] CHH has a male predominance of 3–5 to 1.[4,5] Most patients with CHH are diagnosed late in adolescence or early in adulthood as CHH is challenging to differentiate from other causes of delayed puberty. Effective therapies in both men and women are available for the development of secondary sexual characteristics (virilization and/or estrogenization) and induction of fertility.

A number of important developments in the field have emerged over the past decade. Reversal of CHH occurs in ~10–20% of patients,[6,7] which challenges the dogma that the condition is lifelong. These data have implications for the management of CHH and suggest a plasticity of the GnRH neuronal system. Furthermore, inhibin B, insulin-like 3 (INSL3), anti-Müllerian hormone (AMH) and kisspeptin have emerged as biomarkers for use in the diagnosis and treatment of CHH.[8,9] In addition, hormonal therapies are being personalized to maximize fertility outcomes.[10] Over the past few years, the traditional Mendelian view of CHH as a monogenic disorder has been revised following the identification of oligogenic forms of CHH,[11] which has altered the approach adopted for genetic testing and genetic counselling of patients with CHH. Finally, the availability of next-generation sequencing has started to unravel the complex molecular basis of CHH. This Consensus Statement focuses on the pathogenesis, diagnosis and treatment of CHH in light of recent discoveries and differs from existing guidelines for the treatment of hypogonadism[12–14] as it focuses exclusively on CHH. Definitions of several key terms used in this article are presented in the glossary (Box 1).

Competing interests
The authors declare no competing interests.

Box 1 | Glossary

Anosmia
Complete lack of sense of smell

Cryptorchidism
Maldescended testes that are not in the scrotum (that is, in the inguinal canal or abdomen); can occur in unilateral or bilateral forms

GnRH
GnRH is produced by specialized hypothalamic neurons in a pulsatile manner that stimulates the release of gonadotropins (luteinizing hormone and follicle-stimulating hormone) from the pituitary, which activate gonadal maturation and fertility

Oligogenicity
Mutations in two (or more) CHH genes in one patient

Reversal
Recovery of the hypothalamic–pituitary–gonadal axis (normal circulating levels of sex steroids and/or fertility) after treatment discontinuation

Secondary sexual characteristics
Physical characteristics that result from increased sex hormone production during puberty

Virilization (male)
Change in external genitalia with phallic development, appearance of pubic and/or axillary and/or facial hair, enlarged larynx and/or deepening of voice and increased lean body mass

Estrogenization (female)
Breast development with prominence of nipples, development of pubic and axillary hair, widening of hips and development of labia minora

Abbreviations: CHH, congenital hypogonadotropic hypogonadism; GnRH, gonadotropin-releasing hormone.

Methods

This Consensus Statement is the work of the European consortium studying GnRH biology (COST Action BM1105, http://www.gnrhnetwork.eu/). The COST network includes clinician investigators, geneticists, bioinformaticians, basic scientists and patient advocates from 28 countries. Our recommendations are based on a review of the literature published in the English language between 1990 and 2015 using the following search terms: "congenital hypogonadotropic hypogonadism", "idiopathic GnRH deficiency", "central hypogonadism" and "Kallmann syndrome". The reference lists of identified papers were searched for additional relevant articles. A series of meetings and focused discussions with expert clinicians (specialized in paediatric and adult CHH) were conducted, with a final vetting process involving experts participating in the network from the fields of endocrinology, andrology, genetics and reproductive medicine.

Biology of the GnRH neuronal system

GnRH neurons are unusual neuroendocrine cells as they originate outside the central nervous system in the olfactory placode and then migrate into the brain during embryonic development.[2,3] This route provides a developmental link between the central control of reproduction and the sense of smell, which are both affected in Kallmann syndrome. Data from the past few years suggest that GnRH neurons originate from both the neural crest and ectodermal progenitors, and migrate in close association with growing axons of olfactory and/or terminal nerves.[15,16] Once in the hypothalamus, GnRH neurons detach from their axonal guides, disperse further into the brain parenchyma and stop migrating. At birth, GnRH neurons have reached their final destination in the brain and many of them project into the median eminence, where they release the GnRH decapeptide from their axon terminals into the hypophyseal portal vasculature.[1] GnRH acts via the GnRH receptor, which is expressed on gonadotropic cells in the anterior pituitary gland. This action regulates both synthesis and release of gonadotropins such as luteinizing hormone (LH) and follicle-stimulating hormone (FSH), which control gonadal maturation and adult reproductive physiology via the hypothalamic–pituitary–gonadal (HPG) axis.

Several genes mutated in Kallmann syndrome affect the fate and migration of GnRH neurons. Genes encoding fibroblast growth factor 8 (*FGF8*) signalling pathway proteins,[17–22] chromodomain helicase DNA-binding protein 7 (*CHD7*)[23–27] and sex determining region Y-Box 10 (*SOX10*)[28,29] affect the neurogenic niche in the nasal area and craniofacial development. Conversely, Kallmann syndrome protein, which is now officially known as anosmin 1 (encoded by *KAL1*; following nomenclature change, the gene is now denoted as *ANOS1*),[2] prokineticin-2 and prokineticin receptor 2 (encoded by *PROK2* and *PROKR2*, respectively),[30–33] WD repeat domain 11 (encoded by *WDR11*),[34,35] semaphorin 3A (encoded by *SEMA3A*)[36–38] and FEZ family zinc finger 1 (encoded by *FEZF1*)[39] influence migration of GnRH neurons.

Postmigratory GnRH neurons are embedded in a complex neuronal network of afferents that send information about permissive reproductive cues such as steroid and metabolic hormones to these cells. Individual components of the underlying neural circuits are beginning to emerge. Some key molecules have been discovered through the study of the genetics of CHH.[1] Inactivating mutations in genes encoding kisspeptin-1 (*KISS1*)[40] and its receptor (*KISS1R*)[41,42] halt pubertal development in humans. Extensive experimental studies in various species have demonstrated that kisspeptin-producing neurons are major afferents to GnRH neurons and are essential for different aspects of GnRH function, ranging from the tonic feedback control of GnRH and/or gonadotropin secretion to generation of the pre-ovulatory surge responsible for ovulation.[43] Interestingly, although kisspeptins do not seem to be mandatory for proper GnRH neuron migration, compelling experimental work has documented that populations of kisspeptin neurons undergo a dynamic process of prenatal and postnatal maturation that enables them to establish connections with GnRH neurons early in development[44] (under the control of steroid hormones[45–47]). Similarly, identification of mutations in *TAC3* (encoding tachykinin-3, which is cleaved to form neurokinin-B) and *TACR3* (encoding tachykinin receptor 3; also known as neuromedin-K receptor [NKR])[48–50] in patients with CHH highlights the important role of members of the tachykinin family in the control of GnRH neurons. Furthermore,

these findings led to the identification of a subpopulation of afferent neurons in the arcuate and/or infundibular hypothalamic region, which co-express kisspeptins and NKB.[51] Interestingly, data from the past few years suggest that the actual number of kisspeptin and/or NKB neurons might change during development.[52,53] Mutations in proteins that regulate ubiquitination such as OTU domain-containing protein 4 (encoded by *OTUD4*) and E3 ubiquitin-protein ligase RNF216 (also known as ring finger protein 216; encoded by *RNF216*),[54] as well as in proteins involved in lipid metabolism such as neuropathy target esterase (also known as patatin-like phospholipase domain-containing protein 6; encoded by *PNPLA6*)[55,56] have been identified in patients with Gordon Holmes syndrome (with associated CHH and ataxia). Thus, mutations in these three genes give rise to a broad and progressive neurodegenerative syndrome that includes CHH. In 2014, haploinsufficiency of *DMXL2*, which encodes synaptic protein DmX-like protein 2, was shown to cause a complex new syndrome associating CHH with polyendocrine deficiencies and polyneuropathies.[57]

Peripheral signals that convey information about metabolic status indirectly modulate GnRH neurosecretion. This modulation is well illustrated by the reproductive phenotype of absent pubertal development and hypogonadotropic hypogonadism in patients with inactivating mutations in the genes encoding leptin (*LEP*) or its receptor (*LEPR*).[58] Exactly how the effects of leptin are transmitted to GnRH neurons is unknown, as this neuronal population does not express the leptin receptor.[59] Experimental data suggest that kisspeptin neurons are sensitive to changes in leptin concentrations and metabolic conditions,[43] yet they apparently do not express functional leptin receptor, which indicates the mode of action is indirect.[60] Primary leptin targets for conduction of its reproductive effects probably include nitric oxide-producing neurons in the pre-optic hypothalamus[61] and neuronal circuits in the ventral premammillary nucleus,[60] which might transmit metabolic information to GnRH neurons through kisspeptin dependent and/or independent pathways. Taken together, the identification of genes mutated in the different forms of CHH has facilitated an improved understanding of the neuroendocrine control of reproduction.

Puberty and reproduction

Puberty represents a period of transition from childhood into adulthood during which complete reproductive capacity is attained. Puberty is regulated by the HPG axis. The axis is active *in utero* and shortly after birth[62,63] (a phenomenon referred to as mini-puberty), is subsequently silenced and remains quiescent for years until reawakening at the onset of puberty. Multiple central and peripheral inputs are integrated into pubertal reactivation of the GnRH pulse generator.[64] At the onset of puberty, GnRH-induced pulses of LH are initially nocturnal and, gradually, the pulsatility extends to the daytime as puberty progresses.[65–67]

In boys, FSH stimulates proliferation of immature Sertoli cells and spermatogonia, whereas LH stimulates Leydig cells to produce testosterone. The concerted stimulation of Sertoli cells by FSH and intragonadal testosterone levels 50–100-fold higher than in the systemic circulation leads to the initiation of spermatogenesis. In girls, the early stages of follicular growth are primarily driven by intra-ovarian factors. FSH and LH are needed for follicular maturation, which leads to ovulation. More specifically, LH stimulates theca cells to produce androgens and FSH stimulates recruitment of secondary ovarian follicles and the secretion of estradiol from granulosa cells. Inhibin B and AMH are produced by prepubertal Sertoli cells and granulosa cells.[68] Circulating levels of inhibin B increase during puberty in girls and boys.[69,70] AMH concentrations show only minor fluctuations during female puberty.[71] In boys, AMH concentrations fall as Sertoli cells differentiate and testosterone production increases.[72] In male individuals, INSL3 is secreted by Leydig cells during fetal and immediate postnatal life.[8,73] Testicular INSL3 secretion declines during childhood before increasing at the time of puberty and peaking during adulthood.[74]

Early signs of puberty manifest around the age of 10 years in girls (breast development) and 11.5 years in boys (testicular enlargement and spermarche—appearance of spermatozoa in first morning void).[75–77] Considerable interindividual variation in the timing of pubertal onset exists, ranging from 8 years to 13 years in girls and 9 years to 14 years in boys.[78,79] Subsequent hallmarks of puberty in boys include deepening of the voice and a growth spurt. In girls, menarche occurs at ~12.5 years of age.[78]

Clinical presentation of CHH

Neonatal and childhood

Lack of neonatal activation of the HPG axis can provide early diagnostic cues for identifying CHH at birth or during mini-puberty.[80–83] In male infants, cryptorchidism (that is, maldescended testes) and micropenis can be signs of GnRH deficiency (Figure 1), yet these features are by no means invariable.[84–86] As penile growth occurs during infancy and childhood, micropenis can be assessed using available cross-sectional normative data.[87,88] These two clinical clues warrant hormonal monitoring during mini-puberty. However, more severe genital anomalies, such as hypospadias, point away from a diagnosis of CHH and, instead, indicate a defect of human chorionic gonadotropin (hCG)-driven androgen secretion and/or action in early fetal life, before the initiation of endogenous GnRH activity. No specific clinical signs of CHH present in female neonates. Regardless of sex, when infants are born to parents with CHH, we recommend monitoring reproductive hormones during mini-puberty and performing genetic testing if mutations have been identified in the parents.

Adolescent

The timing and onset of puberty varies widely in the general population.[89] Delayed puberty is classically defined as absence of testicular enlargement (volume <4 ml) in boys by the age of 14 years and absent breast development in girls by the age of 13 years.[90] In addition, use of the stage-line diagram enables the identification of

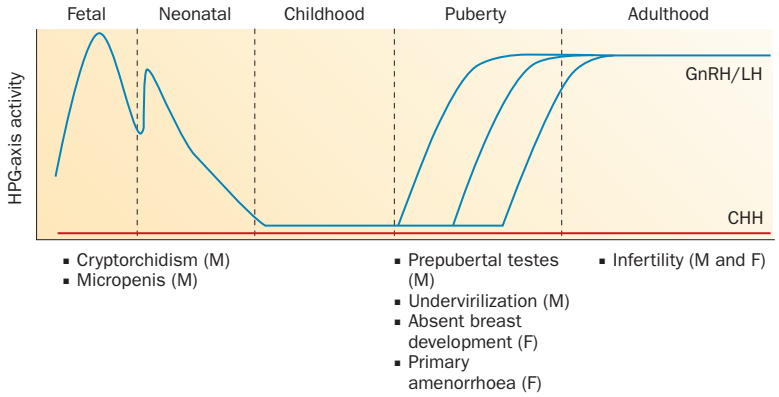

Figure 1 | Activity of the HPG axis across the lifespan. Normal GnRH and LH secretion from fetal life to adulthood (blue line) compared with non-reversible CHH (red line). Reproductive phenotypes observed in CHH are listed under the respective stage of development. Abbreviations: CHH, congenital hypogonadotropic hypogonadism; F, female; GnRH, gonadotropin-releasing hormone; HPG, hypothalamic–pituitary–gonadal; LH, luteinizing hormone; M, male.

patients with delayed puberty, which is based not only on pubertal onset but also on the tempo of pubertal development.[91] In most of these individuals, puberty is initiated and eventually completed (that is, constitutional delay of growth and puberty [CDGP]). By contrast, adolescents with CHH lack pubertal activation of the HPG axis, which leads to absent puberty and infertility (Figure 1). In the majority of patients, puberty never occurs (absent puberty); less commonly, puberty is initiated then arrested (partial puberty).[92–94] Adolescents with CHH exhibit steady linear growth and thus lack the so-called growth spurt.[91,95,96] Given the delayed epiphyseal closure, these individuals often have eunuchoidal proportions. The most common complaints in male adolescents with CHH include absent and/or minimal virilization, low libido and lack of sexual function. Among female adolescents, lack of breast development and/or primary amenorrhoea by the age of 15 years are frequent complaints. In addition, patients with CHH often have low self-esteem, distorted body image, impaired psychosexual development and, in some cases, problems with sexual identity.[97,98] Furthermore, small studies have shown increased anxiety and depression among adolescents with CHH and, as such, these symptoms merit attention.[99,100]

The clinical heterogeneity of CHH makes differentiation from CDGP, which is usually associated with short stature, poor growth velocity and delayed skeletal maturation, difficult.[90,95] The presence of micropenis and/or cryptorchidism argues firmly in favour of CHH, as these features are rarely seen in CDPG. Associated congenital phenotypes are also very useful as they indicate a syndromic form of CHH. The classic, and by far most frequent, form being Kallmann syndrome, which includes anosmia. Other phenotypes like cleft palate or sensorineural deafness also suggest a syndromic form of CHH,[94] most often associated with Kallmann syndrome, but not exclusively so.[18] The clinical sign that points away from CDGP is a poor sense of smell, which suggests Kallmann syndrome.[101–103] Other 'red flags' include congenital hearing impairment with or without pigmentation defects, bimanual synkinesia (mirror movements), dental agenesis, cleft lip and/or palate and cryptorchidism with or without micropenis.[17,28,104–108] Family history indicative of autosomal inheritance cannot be used as a diagnostic tool, as this feature can be observed in both CHH and CDGP.[109]

Adulthood

CHH might be diagnosed after adolescence, when patients present for evaluation of infertility or even for osteoporotic fractures. This delay in diagnosis might be related to missed opportunities to diagnose CHH in adolescence or a reluctance of patients to seek medical evaluation.

Genetics of CHH

CHH is genetically heterogeneous, with both sporadic and familial cases. Several modes of inheritance have been identified, including X chromosome-linked recessive, autosomal recessive and dominant.[1] By examining milder phenotypes (such as delayed puberty or isolated anosmia), the frequency of familial cases increases.[110,111] Understanding of the molecular genetics of CHH and Kallmann syndrome has advanced tremendously in the past 20 years, since the first gene associated with Kallmann syndrome (*KAL1* [*ANOS1*]) was identified by a positional cloning strategy in 1991 (Table 1).[112–115] *KAL1* (*ANOS1*) encodes anosmin-1, a transiently expressed, locally restricted glycoprotein of embryonic extracellular matrices[116] that is involved in fibroblast growth factor (FGF) signalling.[117,118] To date, >25 different genes have been implicated in Kallmann syndrome and/or CHH, which accounts for ~50% of cases.[21] Causative genes for Kallmann syndrome include: *KAL1* (*ANOS1*) in the X-linked form; *FGFR1* (encoding fibroblast growth factor receptor 1),[17,18] *FGF8*,[19,119] *CHD7*,[23–27] *HS6ST1* (encoding heparan-sulphate 6-O-sulphotransferase 1),[20] *SOX10*,[28,29] *SEMA3A* (encoding semaphorin-3A),[36–38] *WDR11* (encoding WD repeat-containing protein 11)[34,35] and *IL17RD* (encoding interleukin-17 receptor D)[21] in the autosomal dominant form; and *PROKR2* and/or *PROK2*,[30–33] and *FEZF1*[39] in the autosomal recessive form, even though it should be noted that most patients carrying mutations in *PROKR2* or *PROK2* carry these mutations in the heterozygous state.[120,121] Genes involved in CHH that are associated with a normal sense of smell include *GNRHR* (encoding gonadotropin-releasing hormone receptor),[122,123] *GNRH1* (encoding gonadotropin-releasing hormone 1),[124,125] *KISS1R*,[41,42] *KISS1*,[40,126] *TACR3* and *TAC3*.[48–50] Other genes such as *FGFR1* or *PROKR2* can be mutated in patients with either Kallmann syndrome or CHH (Table 1). Remarkably, mutations in each Kallmann syndrome or CHH gene identified so far account for <10% of cases.[11,21,127]

CHH has classically been categorized as a monogenic disorder, which means that one defective gene is sufficient to account for the disease phenotype. As such, CHH and Kallmann syndrome caused by mutations in *GNRHR* (a recessive gene) and *KAL1* (*ANOS1*; an X-linked gene),

Table 1 | Genes implicated in CHH

Gene	OMIM	CTO	CHH phenotypes			Overlapping syndromes									
			KS	CHH	CHH reversal	CPHD	CPHD + SOD	WS	CHARGE	HS	SHFM	D-WS	MGS	PEPNS	GHS
KAL1 (ANOS1)	300836	✓	✓	×	✓	×	×	×	×	×	×	×	×	×	×
SEMA3A	614897	✓	✓	×	×	×	×	×	×	×	×	×	×	×	×
SOX10	602229	×	✓	×	×	×	×	✓	×	×	×	×	×	×	×
OL14RD	606807	✓	✓	×	×	×	×	×	×	×	×	×	×	×	×
HESX1	182230	×	✓	×	×	✓	✓	×	×	×	×	×	×	×	×
FEZF1	613301	×	✓	×	×	×	×	×	×	×	×	×	×	×	×
FGFR1	147950	✓	✓	✓	✓	✓	✓	×	×	✓	✓	×	×	×	×
FGF8	612702	✓	✓	✓	×	✓	×	×	×	×	×	×	×	×	×
CHD7	612370	×	✓	✓	✓	×	×	×	✓	×	×	×	×	×	×
FGF17	603725	✓	✓	✓	×	×	×	×	×	×	×	✓	×	×	×
HS6ST1	614880	✓	✓	✓	✓	×	×	×	×	×	×	×	×	×	×
PROK2	610628	✓	✓	✓	×	×	×	×	×	×	×	×	×	×	×
PROKR2	147950	✓	✓	✓	✓	✓	×	×	×	×	×	×	✓	×	×
SEMA7A	607961	✓	✓	✓	×	×	×	×	×	×	×	×	×	×	×
WDR11	614858	✓	✓	✓	×	✓	×	×	×	×	×	×	×	×	×
NSMF	614838	✓	✓	✓	✓	×	×	×	×	×	×	×	×	×	×
AXL	109135	×	✓	✓	×	×	×	×	×	×	×	×	×	×	×
GNRH1	614841	×	×	✓	×	×	×	×	×	×	×	×	×	×	×
GNRHR	146110	✓	×	✓	✓	×	×	×	×	×	×	×	×	×	×
KISS1	614842	×	×	✓	×	×	×	×	×	×	×	×	×	×	×
KISS1R	614837	✓	×	✓	×	×	×	×	×	×	×	×	×	×	×
TAC3	614839	✓	×	✓	✓	×	×	×	×	×	×	×	×	×	×
TACR3	614840	✓	×	✓	✓	×	×	×	×	×	×	×	×	×	×
LEP	614962	×	×	✓	×	×	×	×	×	×	×	×	×	×	×
LEPR	614963	×	×	✓	×	×	×	×	×	×	×	×	×	×	×
PCSK1	162150	×	×	✓	×	×	×	×	×	×	×	×	×	×	×
DMXL2	616113	×	×	✓	×	×	×	×	×	×	×	×	×	✓	×
RNF216	609948	×	×	✓	×	×	×	×	×	×	×	×	×	×	✓
OTUD4	611744	×	×	✓	×	×	×	×	×	×	×	×	×	×	✓
PNPLA6	603197	×	×	✓	×	×	×	×	×	×	×	×	×	×	✓
NR0B1	300200	×	×	✓	×	×	×	×	×	×	×	×	×	×	×

Abbreviations: CHH, congenital hypogonadotropic hypogonadism; CHARGE, coloboma, heart defects, atresia of choanae, retardation of growth and/or development, genital and/or urinary defects, ear anomalies or deafness; CPHD, combined pituitary hormone deficiency; CTO, contributes to oligogenicity; D-WS, Dandy-Walker syndrome; GHS, Gordon Holmes syndrome; HS, Hartsfield syndrome; KS, Kallmann syndrome; MGS, Morning Glory syndrome; OMIN, online Mendelian inheritance in man; PEPNS, polyendocrine deficiencies and polyneuropathies; SHFM, split-hand/foot malformation; SOD, septo-optic dysplasia; WS, Waardenburg syndrome.

respectively, are highly penetrant,[127] yet such a pattern is not observed for all genes involved in CHH and/or Kallmann syndrome. For instance, the p.Arg622X *FGFR1* mutation has been reported in unrelated Kallmann syndrome and CHH probands (Figure 2). Examination of pedigrees reveals low penetrance for most CHH genes and variable expressivity among affected individuals carrying the same gene defect. Such an observation led to the hypothesis that multiple gene defects could synergize to produce a more severe CHH phenotype (that is, oligogenicity).[30,128] Studies in large CHH cohorts indicate that at least 20% of cases are oligogenic[11,21] (Table 1). This genetic model might be useful to predict genotype–phenotype correlations.[5] Interestingly, genome-wide association studies have identified >100 loci affecting the age of menarche,[129] which confirms a genetic contribution to pubertal timing. Surprisingly, little overlap exists between the genes implicated in age at menarche and CHH.[129]

Diagnosis of CHH

Central to the evaluation process for diagnosing CHH is the exclusion of differential diagnoses such as pituitary tumour or functional causes (Box 2).

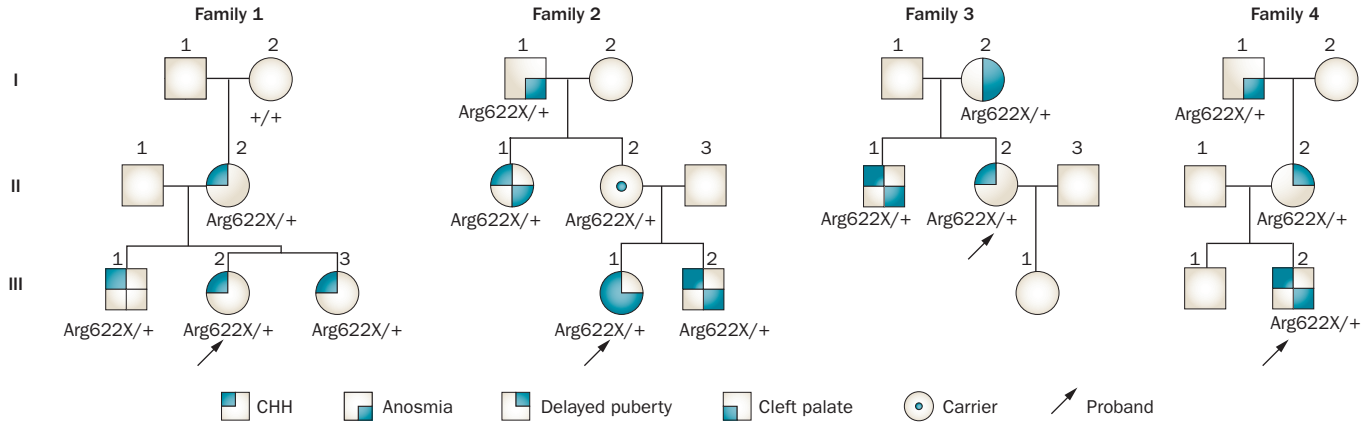

Figure 2 | *FGFR1* mutation p.Arg622X in an autosomal dominant form of CHH with incomplete penetrance and variable expressivity. In family 1, the CHH phenotype is fully penetrant.[147] In the three other pedigrees, family members carrying the same *FGFR1* mutation have variable reproductive and olfactory phenotypes and family 2 includes an asymptomatic carrier.[17,148] + denotes wild-type allele. Abbreviation: CHH, congenital hypogonadotropic hypogonadism.

Clinical, biochemical & imaging investigations

The mini-puberty provides a brief opportunity to identify CHH. For male infants, cryptorchidism with or without micropenis can be suggestive of CHH (Figure 1).

Box 2 | Forms of CHH and differential diagnosis

Forms of CHH

GnRH deficiency and defective sense of smell
- Kallmann syndrome

Isolated GnRH deficiency (normal sense of smell)
- Normosmic CHH

Complex syndromes including CHH or KS
- Combined pituitary hormone deficiency
- Septo-optic dysplasia
- CHARGE syndrome
- Adrenal hypoplasia congenita with HH
- Waardenburg syndrome
- Bardet–Biedl syndrome
- Gordon Holmes syndrome
- Others

Differential diagnosis

Functional causes
- Malnutrition and/or malabsorption
- Any chronic disease (for example, IBS or asthma)
- Coeliac disease
- Eating disorders
- Excessive exercise

Systemic causes
- Haemochromatosis
- Sarcoidis
- Histiocytosis
- Thalassaemia

Acquired causes
- Pituitary adenomas and/or brain tumours
- Rathke's cleft cyst
- Pituitary apoplexy
- Radiation (brain or pituitary)
- Medication induced (such as, by steroids, opiates or chemotherapy)

Abbreviations: CHARGE, coloboma, heart defects, atresia of choanae, retardation of growth and/or development, genital and/or urinary defects, ear anomalies or deafness; CHH, congenital hypogonadotropic hypogonadism; GnRH, gonadotropin-releasing hormone; HH, hypogonadotropic hypogonadism; IBS, irritable bowel syndrome; KS, Kallmann syndrome.

In such cases, hormone profiling at 4–8 weeks of life can be used to make a diagnosis[83,85] based on comparisons with established reference ranges for gonadotropins, sex steroids and inhibin B levels.[62,63,70,130] Female neonates born to parents with CHH should undergo biochemical evaluation; serum levels of FSH during the mini-puberty seem to be the most sensitive indicator of CHH in this setting.[131] During childhood, diagnosis of CHH is very challenging as this interval is a physiologically hypogonadal period of development. Undetectable levels of FSH (but not of LH) or absence of response to a GnRH test in childhood might indicate CHH.

CHH diagnosis is typically made during adolescence or early adulthood (Table 2). Approximately half of patients with CHH have Kallmann syndrome.[101] Self-report of anosmia is reliable, yet claims of having a 'good' sense of smell do not always align with formal smell testing. Most patients with Kallmann syndrome display olfactory bulb hypoplasia and/or aplasia on brain MRI; however, this finding is not fully concordant with sense of smell.[102] An assessment of family history is important to identify familial cases. A family history of CDGP does not exclude CHH as pedigrees of patients with CHH seem to be enriched in delayed puberty, subfertility and anosmia or hyposmia.[132] A detailed physical examination is needed, with particular attention paid to genitalia (that is, measurement of testicular volume using a Prader orchidometer) and virilization and/or estrogenization (that is, Tanner staging for breast development and pubic hair[133,134]). Eunuchoidal proportions are often present.[135] A stage-line diagram is useful for providing developmental stages ±SD or as percentiles, as well as the tempo of pubertal development ±SD per year.[91] Additionally, signs of associated developmental anomalies such as cryptorchidism with or without micropenis, midline defects or signs suggestive of combined pituitary hormone deficiency should be evaluated (Box 2).

Biochemical evaluation includes a variety of tests to exclude other causes of CHH such as pituitary tumour or functional causes (Table 3). Isolated hypogonadotropic

Table 2 | Clinical evaluation of CHH

Assessment	Signs	Focus
Growth	Evaluation of growth (growth chart)	Linear growth, acceleration and/or deceleration in growth
	Eunuchoidal proportions	Closure of epiphysis
Sexual	Cryptorchidism and micropenis	Severe CHH in male individuals
	Sexual development (Tanner staging of pubic hair, penis and gonads in male individuals and breasts in female individuals)	Puberty (Tanner stage 2 genitalia in male individuals indicates the onset of puberty)
	Male individuals: testicular volume (Prader orchidometer) and penile length	Male individuals: at age 14 years, testicular volume <4 ml indicates absent puberty Assess micropenis (versus cross-sectional normative data)
	Female individuals: breast development and age at menarche	Female individuals: at age 13 years, Tanner stage 1 breasts indicate absent puberty Primary amenorrhoea by age 15 years
Other	Olfaction (using odorant panel and/or olfactometry)	Hyposmia and/or anosmia
	Snellen eye chart, ocular fundoscopy	Optic nerve hypoplasia
	External ear shape and audition	Hearing impairment
	Number of teeth	Cleft lip and/or palate, missing tooth or teeth
	Pigmentation of skin and hair	Achromic patches
	Rapid sequential finger–thumb opposition	Bimanual synkinesis Neurodevelopmental delay

Abbreviation: CHH, congenital hypogonadotropic hypogonadism.

hypogonadism is characterized by low serum levels of testosterone in male individuals (usually <2 nmol/l) in the setting of low or normal serum levels of gonadotropins (LH and FSH) with otherwise normal pituitary function (Figure 3). In female individuals, serum levels of estradiol are often low, sometimes undetectable in the setting of low-normal gonadotropin levels. Serum levels of estradiol seem to correlate with breast development, as most women with absent breast development have very low or undetectable levels, whereas women with breast development exceeding Tanner stage B2 usually have measurable serum levels of estradiol.

In early adolescence (13–16 years), a diagnosis of CHH is challenging to make as isolated hypogonadotropic hypogonadism is common to both CHH and CDGP. In CHH, the GnRH stimulation test has a poor diagnostic value. In the absence of a gonadotropin response, this test can help to confirm severe GnRH deficiency, yet clinical signs are often already evident (that is, prepubertal testes or absence of breast development). By contrast, the GnRH stimulation test rarely enables differentiation of CDGP from partial forms of CHH—the most challenging differential diagnosis. A number of other tests have been proposed to differentiate between these two entities, including a combination of GnRH and hCG stimulation tests, as well as measurement of serum levels of inhibin B, AMH or INSL3.[74,136–142] However to date, no gold-standard diagnostic test exists to fully differentiate CHH from CDGP.[143]

Inhibin B is a marker of Sertoli cell number and correlates with testicular volume.[92] In men with CHH and severe GnRH deficiency, serum levels of inhibin B are typically very low (<30 pg/ml; Figure 3). However, for partial forms of CHH, inhibin B levels overlap with those in patients with CDGP and in healthy controls.[138,139] INSL3 is a good marker of Leydig cell function[144] and, as such, levels of INSL3 are typically low in male individuals with CHH (at diagnosis or on testosterone therapy) and increase with LH and/or hCG stimulation.[74] However, further studies are needed to identify the potential clinical utility of INSL3 measurements, such as differentiating CHH from CDGP or as a predictor of reversible CHH. Kisspeptin is a potent stimulator of GnRH-induced LH secretion.[9] Exogenous kisspeptin administration failed to induce a response in patients with CHH regardless of genotype. However, a male individual with reversible CHH exhibited a robust response to exogenous kisspeptin[145] and continuous kisspeptin infusion was demonstrated to overcome genetic defects in the kisspeptin signalling pathway.[146] In terms of clinical application, kisspeptin has been used with therapeutic effect in women with hypothalamic amenorrhoea,[147] as well as for egg maturation during assisted fertility.[148] Thus, although still investigational, kisspeptin seems to be emerging as useful in several therapeutic areas.

CHH work-up also includes cranial MRI to assess for tumours and/or space occupying lesions and to visualize inner ear and olfactory structures, bone age in adolescents to compare with chronological age and to assess epiphyseal closure, abdominal and/or pelvic ultrasonography to assess unilateral renal agenesis and to visualize the ovaries and/or uterus or testis, and bone densitometry (dual-energy X-ray absorptiometry) to assess bone density.

Genetic testing

Genetic testing is useful for diagnosis, prognosis[149] and genetic counselling in CHH.[127] The first step in prioritizing genes for genetic testing is to establish the inheritance pattern. Briefly, X-linked inheritance is observed in pedigrees when male-to-male transmission of the disease phenotype does not occur but the disease is observed in male members of the maternal line. This feature is typical for *KAL1* (*ANOS1*) mutations underlying Kallmann syndrome.[150] Autosomal dominant inheritance is observed in a family with vertical transmission of the disease phenotype across one or more generations. In this setting, male and female individuals are equally affected and male-to-male transmission can be observed. Importantly, incomplete penetrance and variable expressivity of the disease among people with identical mutations can be observed for most CHH genes (Figure 2).[17,151,152] In autosomal recessive inheritance, vertical transmission of the disease phenotype does not occur and ~25% of offspring are affected. Examples of autosomal recessive forms include those involving mutations in *GNRHR*, *KISS1R*, *TACR3*, *PROKR2* or *FEZF1* (Table 1).

Genetic testing can also be guided by the presence of additional phenotypic features. Cleft lip and/or palate

Table 3 | Biochemical, radiological and genetic evaluation of CHH

Assessment	Marker	Focus
Biochemical	0800–1000 h hormonal measurements (LH, FSH, testosterone or estradiol, prolactin, free T_4, TSH, cortisol, IGF-1 and IGFBP3)	Assess for combined pituitary hormone deficiency Demonstrate isolated hypogonadtropic hypogonadism
	Specialized endocrine tests (serum inhibin B, AMH, INSL3, GnRH and/or hCG stimulation test)	Evaluate the degree of GnRH deficiency and function of the gonads
	Spermiogram	Assess fertility
	Liver and/or renal function and inflammatory markers (LFTs, BUN, lytes, CBC, Ca, CRP, ESR and faecal calprotectin)	Assess systemic and/or inflammatory disease
	Screen for coeliac disease	Assess malabsorption
	Iron overload screen (ferritin, TIBC% saturation)	Assess juvenile haemochromatosis
Radiologic	Wrist radiography (bone age)	Delayed bone age (chronological age greater than bone age)
	Brain MRI	Exclude hypothalamo–pituitary lesion Assess olfactory bulbs and sulci Visualize optic nerve Assess inner ear (semicircular canals)
	Renal ultrasonography	Rule out unilateral renal agenesis
	Bone density (DXA)	Assess osteopenia and/or osteoporosis
Genetic	Comparative genomic hybridization array or karyotype	Large, rare deletions and/or insertions to identify contiguous gene syndromes
	Gene screening (Sanger or next-generation sequencing)	Mutations in CHH and/or KS genes

Abbreviations: AMH, anti-Müllerian hormone; BUN, blood urea nitrogen; CBC, complete blood count; CHH, congenital hypogonadotropic hypogonadism; CRP, C-reactive protein; DXA, dual-energy X-ray absorptiometry; ESR, erythrocyte sedimentation rate; FSH, follicle-stimulating hormone; GnRH, gonadotropin-releasing hormone; hCG, human chorionic gonadotropin; IGF-1, insulin growth factor 1; IGFBP3, insulin-like growth factor-binding protein 3; INSL3, insulin-like 3; KS, Kallmann syndrome; LFTs, liver function tests; LH, luteinizing hormone; TIBC, total iron-binding capacity.

and skeletal anomalies indicate mutations in genes encoding components of the FGF8 signalling pathway (for example, *FGFR1*, *FGF8* and *HS60ST1*).[17,20,21,104,153] Signs associated with CHARGE syndrome (CHARGE: coloboma, heart defects, atresia of choanae, retardation of growth and/or development, genital and/or urinary defects, and ear anomalies and/or deafness) favour screening for mutations in *CHD7*.[24–26,104] Likewise, the association of CHH with congenital hearing impairment should direct screening towards *CHD7*, *SOX10* and/or *IL17RD*.[21,28,104] Bimanual synkinesia or renal agenesis are important clinical cues suggestive of *KAL1* (*ANOS1*) mutations.[101,104,114,154,155] Early onset of morbid obesity associated with CHH suggests mutations in *LEP*, *LEPR* or *PCSK1*.[58,156] Indeed, combining CHH with specific associated phenotypes can increase the probability of finding causal mutations by targeted gene sequencing (Figure 4).[21,28,104,157] CHH accompanied by other syndromic features such as congenital ichthyosis[158] or spherocytosis[159] is suggestive of a contiguous gene syndrome for which a karyotype or comparative genomic hybridization array analysis might be useful for identifying underlying chromosomal abnormalities.[159–161]

Genetic counselling

A legitimate question often raised by patients with CHH relates to the risk of passing on the disease to their offspring.[26,127,162] When autosomal dominant transmission is suspected, the patient and partner should receive genetic counselling before fertility-inducing treatment. This counselling should include clear explanation of the 50% theoretical risk of transmitting the disease. In such cases, hormonal profiling during mini-puberty should be conducted. In proven autosomal recessive forms, which affect both male and female individuals, the risk of disease transmission to offspring is very low in the absence of consanguinity (that is, inter-related parents). This reduced risk is due to the very low frequency of heterozygous healthy carriers in the general population, which should be reassuring for intended parents. Male individuals with X-linked Kallmann syndrome should be informed of the automatic transmission of the mutation to their unborn daughters (obligate carriers) and the necessity for genetic counselling of these girls after the age of puberty. When patients carry several mutations in different CHH or Kallmann syndrome genes (that is, oligogenicity), genetic counselling is difficult and the transmission risk is variable.[127] Finally, the advent of next-generation sequencing will probably improve the molecular genetic diagnosis of CHH.[163] More specifically, this technology will help identify cases of oligogenicity and might promote personalized approaches to genetic counselling.

Treatment of CHH

No treatments exist for many of the CHH-associated phenotypes such as anosmia, bimanual synkinesia or renal agenesis. Others phenotypes such as cleft lip and/or palate, hearing loss or various skeletal defects require surgical and/or specialist intervention early in life. The approach to CHH treatment is largely determined by goals such as developing only virilization or estrogenization, or inducing fertility as well.

During infancy and childhood

In affected boys, the focus of most treatment is on appropriate testicular descent and penile growth. Cryptorchidism (particularly bilateral) can have far reaching negative effects on future fertility potential.[164,165] As such, current recommendations advocate surgical correction at 6–12 months.[166–168] Micropenis should be treated early using short-term, low-dose testosterone (dihydrotestosterone or testosterone esters) to induce penile growth (Table 4).[86,169] Importantly, as the duration of treatment is brief, no major concerns regarding virilization or the development of secondary sexual characteristics exist; however, erections might be observed. Gonadotropins (LH and FSH) have been used to treat patients with micropenis and evidence of absent minipuberty.[170,171] Such an approach might be beneficial for the additional stimulatory effect on gonadal development. Indeed, treatment with FSH stimulates proliferation of immature Sertoli cells and spermatogonia in men with CHH before pubertal induction.[10] Furthermore,

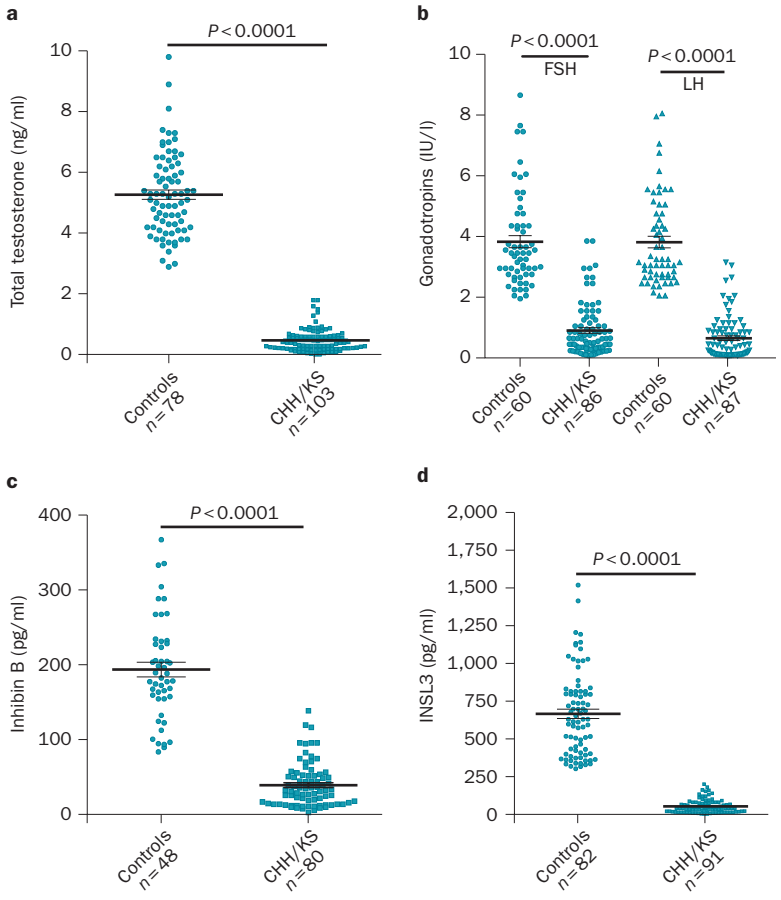

Figure 3 | Hormonal profile of patients with CHH and/or KS compared with healthy controls. **a** | Serum testosterone levels. **b** | Serum gonadotropin (LH and FSH) levels. **c** | Serum inhibin B levels. **d** | Serum INSL3 levels. Healthy male individuals (controls; aged 17—27 years) versus untreated patients with CHH and/or KS (aged 17–29 years). Abbreviations: CHH, congenital hypogonadotropic hypogonadism; FSH, follicle-stimulating hormone; INSL3, insulin-like 3; LH, luteinizing hormone; KS, Kallmann syndrome. Adapted with permission from The Endocrine Society © Trabado, S. *et al. J. Clin. Endocrinol. Metab*. **99**, E268–E275 (2014) and Young, J. *J. Clin. Endocrinol. Metab*. **97**, 707–718 (2012).

neonatal gonadotropin therapy can increase intratesticular testosterone concentrations without fear of inducing spermatogenesis or negatively affecting the ultimate number of Sertoli cells (an important determinant of fertility), as Sertoli cells do not express the androgen receptor during the first 5 years of life.[172,173] Although initial studies involving neonatal administration of gonadotropin have been promising, these studies are limited, both in number of studies and number of participants, and further investigations are needed to examine the effectiveness of gonadotropin and its effect on long-term outcomes such as fertility (Box 3).

During adolescence and adulthood

The aim of treatment is to induce virilization or estrogenization and normal sexual function, to stimulate statural growth, to promote bone health and to address concerns about future fertility as well as psychological and emotional wellbeing.[174,175] Sex steroid therapy (that is, testosterone in male individuals and estradiol before estradiol + progesterone in female individuals) induces the characteristic changes of puberty, which includes psychosocial development (Table 4).

Induction of male sexual characteristics

Virilization is typically the primary objective of treating CHH[174] in order to diminish the psychological suffering caused by sexual infantilism.[97,98,176] Accordingly, prompt initiation of therapy after diagnosis is important and usually involves an injectable testosterone ester (aromatizable androgen such as enanthate, cypionate or undecanoate) or transdermal testosterone application with the precise protocol depending on the age at diagnosis and local practice.[94,177,178] Paediatric endocrinologists treating young patients (that is, from 12 years of age) usually begin treatment with low-dose testosterone (for example, 50 mg of testosterone enanthate monthly, 10 mg transdermal testosterone every second day, or 40 mg oral testosterone undecanoate daily) and gradually increase to full adult dosing over the course of 18–24 months, depending on age at the start of treatment. Such regimens help mimic natural puberty and maximize statural growth while affording time for psychosexual development and minimizing the risk of precocious sexual activity.[95,174]

Adult endocrinologists often see patients with CHH in late adolescence or early adulthood when the main complaint is the lack of pubertal development.[94] In such cases, the therapeutic approach is often more incisive than for younger patients, involving higher initial testosterone doses (200 mg testosterone enanthate monthly, then every 2–3 weeks) than those used by paediatric endocrinologists to induce more rapid virilization (Box 4). The frequency of injections should be guided by trough serum testosterone measurement targeting the lower end of the normal range to avoid periods of hypogonadism between injections. Of note, testosterone treatment will not induce gonadal maturation or fertility in these patients.[94,177] Importantly, increased testicular volume following testosterone treatment is an indicator of reversal[6,7,179–185] and requires re-assessment of the HPG axis in the absence of testosterone replacement therapy. An estimated 10–20% of patients with CHH undergo spontaneous recovery of reproductive function,[6,7] and this includes patients with mutations in known CHH genes (Table 1). Moreover, reversal is not always lasting[7,184] and, as yet, no predictors to identify those who will reverse or relapse exist (Box 3). Ongoing monitoring is thus justified.

Virilization in adolescents or young adults can also be achieved with pulsatile GnRH[186–188] or gonadotropin therapy (hCG alone or combined with FSH).[189,190] These fertility-inducing treatments stimulate endogenous testosterone (and estradiol) production by testicular Leydig cells and are associated with good physical and psychological outcomes.[191] In clinical practice, testosterone enanthate is the most frequently used treatment for young adults, yet clinicians should avoid adherence to an overly dogmatic approach. Few published studies have examined the effects of treatment to induce

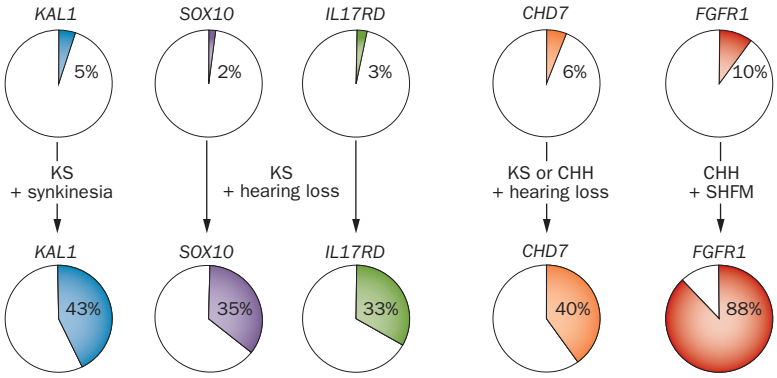

Figure 4 | Combined phenotypes can facilitate identification of the causal gene in CHH. Examples shown for *KAL1* (*ANOS1*),[104] *SOX10*,[28] *IL17RD*,[21] *CHD7*[104] and *FGFR1*.[157] Abbreviations: CHH, congenital hypogonadotropic hypogonadism; KS, Kallmann syndrome; SHFM, split-hand/foot malformation.

virilization, as well as fertility treatment, on quality of life and sexuality in men with CHH,[97,98,192] particularly in those with severe CHH (micropenis and cryptorchidism). Regardless of the therapy considered, patients and their entourage (that is, spouse, partner or family) should be clearly informed that treatment is likely to be lifelong and to require regular monitoring for optimal benefit.

Induction of male fertility

CHH manifests as a lack of gonadal development and infertility. Importantly, CHH is one of the rare treatable causes of male infertility. Spermatogenesis can be induced either by long-term pulsatile GnRH administration (pump)[193] or more commonly by subcutaneous gonadotropin injections (2–3 times weekly).[194–198] These therapies induce both testicular testosterone production by Leydig cells and spermatogenesis in the seminiferous tubules.[199–202] The majority of patients with CHH develop sperm in their ejaculate with long-term therapy.[149,193,196,197] Although these treatments seem to have similar fertility outcomes,[199–202] comparing their efficacy is difficult owing to the small numbers of patients studied, heterogeneity in terms of degrees of GnRH deficiency (testicular volume before treatment), prior treatment and a lack of randomized studies performed to date.

A number of predictors of fertility outcome have emerged in CHH. First, cryptorchidism indicates a poor fertility prognosis.[193,195] Men with CHH who have cryptorchidism often require extended courses of treatment (18–24 months).[203,204] Prepubertal testicular volume (<4 ml) and/or low serum levels of inhibin B are also negative determinants of fertility outcome.[193–197] Clinicians should be aware that although ~75% of men will develop sperm, counts rarely reach the normal range with long-term therapy. A 2014 meta-analysis identified a mean sperm count of 5.9×10^6/ml (range: $4.7–7.1 \times 10^6$/ml) for gonadotropin therapy and 4.3×10^6/ml (range: $1.8–6.7 \times 10^6$/ml) for GnRH therapy.[200] However, an important feature of patients with CHH is that low sperm concentrations do not preclude fertility.[205]

In clinical practice, when testicular volume is <4 ml, the classic treatment is either pulsatile GnRH (25 ng/kg every 2 h, titrated for trough serum testosterone level) or combined gonadotropin therapy (hCG: 1,000–1,500 IU + FSH: 75–150 IU 2–3 times weekly, based on available formulations). In patients with some gonadal development (testicular volume >4 ml) and no history of cryptorchidism, induction of spermatogenesis can be achieved with hCG monotherapy.[194,206–208] If no sperm is present in the ejaculate after 3–6 months of treatment, FSH can be used to stimulate the seminiferous tubules.[198] In the most severely affected men with CHH (testicular volume <4 ml), a sequential treatment has emerged in an attempt to maximize fertility potential.[10,209,210] The unopposed FSH treatment before maturation by hCG (or alternatively, GnRH-induced LH) stimulates proliferation of immature Sertoli and germ cells. Outcomes of patients treated with this regimen demonstrate successful induction of testicular development and fertility in men with CHH who have prepubertal testes.[10,210] To definitively determine the optimal approach to fertility treatment in men with CHH and severe GnRH deficiency (with or without cryptorchidism), an international, multicentre randomized trial is needed (Box 3).

Assessment of the fertility status of the female partner is crucial before induction of spermatogenesis and includes a detailed reproductive and/or menstrual history and evaluation of the integrity of the uterine cavity and tubal permeability (by use of sonohysterosalpingography or hysterosalpingography). Anovulation and ovarian reserve can be assessed via a combination of history of menstrual cycle abnormalities, laboratory examination and pelvic ultrasonography. Couples should be counselled appropriately on the basis of the identified predictors of CHH fertility-inducing treatment,[193–198,200] as well as the partner's reproductive status and age in order to develop realistic expectations regarding treatment outcome.

For men with CHH who have severely impaired sperm counts (or quality), assisted reproductive technology (ART) treatments such as *in vitro* fertilization can be used to improve fertility. More invasive procedures than ART include surgical testicular sperm extraction followed by *in vitro* intracytoplasmic sperm injection (ICSI). ICSI was initially used in men with CHH as a means to shorten the duration of treatment;[211] however, outcomes are improved after maximal testicular volume is attained. Overall, fertilization is achieved in 50–60% of cases of ICSI, with pregnancy occurring in about a third of cycles.[212–215] Although a study of sperm quality in men with CHH who received fertility-inducing treatment revealed neither altered DNA integrity nor an increased risk of chromosomal abnormalities,[216] the possibility of an increased risk of birth defects with ART remains controversial. Once pregnancy is achieved, men with CHH can be transitioned to testosterone replacement therapy (injectable or transdermal preparations) for long-term treatment. Our consensus is that treatment be continued through the first trimester of pregnancy, in order to maintain male fertility capability in

Table 4 | Approach to treatment of CHH

Target population	Goals	Treatment	Clinical monitoring	Laboratory monitoring	Comments
Neonatal and childhood					
Male individuals with cryptorchidism with or without micropenis	Testicular descent	Cryptorchidism: orchidopexy (patients aged <1 year)	Testicular volume	Testosterone, LH, FSH, inhibin B and AMH levels	Baseline levels of testosterone, LH and FSH between days 14–90; for later timepoints, measure after GnRH agonist stimulation No current indication for female individuals
	Penis growth	Micropenis: testosterone, DHT or gonadotropin therapy (patients aged 1–6 months)	Penile growth		
Adolescent					
Patients aged 14–15 years (earlier in the presence of specific signs such as anosmia)	Virilization or estrogenization Sexual function Growth and bone health Gonadal maturation and future fertility Psychological wellbeing	Male patients: testosterone (oral, injectable or transdermal) Gonadotropins?	Genital development Growth and epiphyseal closure Virilization Sexual function Wellbeing Adherence Reversibility	Morning serum testosterone levels (trough levels for injections), LH, FSH and inhibin B levels, haemocrit	Testosterone treatment will not induce testicular growth or fertility
		Female patients: estradiol (oral or transdermal) followed by estradiol + progesterone or progestin	Breast development Growth and epiphyseal closure Estrogenization Feminized body Menses Sexual function Bone health Wellbeing Adherence Reversibility	No specific	Estradiol must be increased slowly before the combination of estradiol + progesterone or progestin to maximize breast development and avoid areolar protrusion
Adulthood					
All patients	Sexual function Fertility Limiting comorbidities Psychological wellbeing Puberty induction	Male patients: Testosterone (injectable or transdermal) hCG ± FSH FSH, FSH + hCG GnRH pump	Pubertal development Sexual function and libido Bone health Wellbeing Adherence Fertility Reversibility	Tough serum testosterone levels, LH, FSH and inhibin B levels, haematocrit, PSA levels	Sex steroid replacement will not induce fertility
		Female patients: Estradiol (oral or transdermal) Progesterone or progestin FSH + hCG or GnRH pump	Pubertal development Sexual function and libido Bone health Wellbeing Adherence Fertility Reversibility	Serum levels of estradiol, LH, FSH, inhibin B and AMH	Sex steroid replacement will not induce fertility

Abbreviations: AMH, anti-Müllerian hormone; CHH, congenital hypogonadotropic hypogonadism; DHT, dihydrotestosterone; FSH, follicle-stimulating hormone; GnRH, gonadotropin-releasing hormone; hCG, human chorionic gonadotropin; LH, luteinizing hormone; PSA, prostate-specific antigen.

the event of miscarriage. Furthermore, although previous gonadotropin cycles seem to shorten the time to fertility in subsequent treatments,[196] fertility in the future is not guaranteed. We, thus, recommend cryopreserving sperm once fertility has been attained.

Induction of female sexual characteristics

Puberty can be induced by oral or preferably transdermal estradiol administration in girls. The use of specific low-dose estradiol formulations (for example, 0.1 mg daily) are important.[174] Transdermal estradiol administration is started (from age 10 years at the earliest) at low doses (typically 0.05–0.07 μg/kg nocturnally), with the goal of mimicking estradiol levels during gonadarche. In older girls, when breast development is a high priority, the starting dose of transdermal estradiol can be 0.08–0.12 μg/kg.[217] The estradiol dose is slowly increased over 12–24 months, after which time cyclic gestagen is added (or after the first menstrual bleed) in order to maximize breast development. In adulthood, estradiol is typically given orally (at a dose of 1–2 mg) or transdermally (50 μg daily by patch or 1–2 pumps of 0.06% gel daily) as a maintenance dose with a cyclic progestin regimen (progesterone 200 mg for 14 days of the cycle, depending on formulation) to avoid endometrial hyperplasia. In the majority of women with CHH, estroprogestin therapy is effective in inducing harmonious development of the breasts and genitals, as well as an increased sense of femininity that contributes to a satisfactory emotional and sexual life. Estrogen treatment increases uterine size and combined estrogen and progestin therapy induces monthly withdrawal bleeding, but does not induce ovulation. For fertility, gonadotropins or GnRH therapy are necessary and effective.

Box 3 | Challenges for management of CHH and goals

Detection and diagnosis of CHH
- Discover novel biomarkers for CHH diagnosis and assess the utility of refining the phenotype to enhance genetic testing

Best treatment and timing of initiating treatment
- Long-term studies evaluating: neonatal gonadotropin treatment to optimize fertility; gonadotropin treatment during adolescence versus adulthood for fertility optimization; and role of prior androgen treatments on fertility outcomes

Adherence to treatment and transition to adult services
- Interventions to promote adherence and structured transitional care from paediatric to adult services

Psychological distress and psychosexual effect
- Targeted psychosocial interventions and enhanced peer-to-peer support

Specialized care for fertility induction
- International, multicentre trials to identify optimal approach to treatment

Genetic counselling (monogenic versus oligogenic forms of CHH)
- Personalized counselling of patients using results obtained by next-generation sequencing technologies

Abbreviation: CHH, congenital hypogonadotropic hypogonadism.

Box 4 | Goals for testosterone replacement therapy*

Promote
- Virilization (for example, axillary, body and facial hair)
- Development of muscle mass and strength
- Pubertal growth spurt
- Skeletal maturation and bone mass
- Deepening of voice
- Growth of penis
- Libido and sexual function
- Self-confidence and wellbeing

Monitor and minimize
- Acne
- Gynaecomastia
- Aggressiveness
- Polycythaemia

*In male patients with congenital hypogonadotropic hypogonadism.

Induction of female fertility

Infertility in women with CHH is related to insufficient follicular maturation, which leads to chronic anovulation.[218] GnRH-induced pituitary gonadotropins induce absent or decreased physiological ovarian stimulation; however, no evidence of a decreased follicular reserve exists. This point must be emphasized as decreased circulating AMH concentrations observed in female patients with CHH and severe GnRH deficiency could wrongly suggest an alteration in ovarian reserve and therefore a poor fertility prognosis.[219] By contrast, women with CHH should be informed that their infertility is treatable and that their reproductive prognosis is quite good in the absence of a male factor of infertility.

Before considering ovulation induction, sonohysterosalpingography or hysterosalpingography should be performed to evaluate the integrity of the uterine cavity and fallopian tubes. One must also ensure regular sexual intercourse takes place and rule-out an associated male factor in infertility by semen analysis.[220] Couples should be advised on the optimal timing of sexual intercourse during the ovulation induction as this first-line therapy does not require *in vitro* fertilization.[221] The goal of ovulation induction therapy in female patients with CHH is to obtain a single ovulation and to avoid multiple pregnancies. Ovulation can be achieved either with pulsatile GnRH therapy[222–224] or, alternatively, with FSH treatment followed by hCG or LH to trigger ovulation.[225] One caveat is that most women with CHH do not secrete endogenous LH and, thus, require a complement of LH to stimulate local production of androgen substrates by theca cells, which facilitates sufficient secretion of estradiol by the dominant follicle.[226–228] Physiologic estradiol secretion promotes optimal endometrial development and the cervical mucus production needed for sperm transit and embryo implantation.[229] Typically, subcutaneous FSH doses of 75–150 IU per day are sufficient.[223–225,229] The usual time required to obtain a dominant mature follicle (>18 mm) is ~12 days.[223–225,229] The starting dose of FSH is often increased or decreased depending on the ovarian response, as assessed by repeated serum estradiol measurements or by counting maturing follicles (using ultrasonography) performed approximately every 3–4 days. This regimen helps to minimize multiple pregnancies and the risk of ovarian hyperstimulation syndrome.[230] After ovulation, progesterone production can be stimulated by repeated hCG injections or direct administration of progesterone during the postovulatory phase, until embryonic endogenous hCG secretion takes over.

Ovulation induction with a pulsatile GnRH pump can be effective even in the presence of GnRH resistance, such as in women with CHH who have mutations in *GNRHR*.[231] GnRH can be administered at different pulse frequencies across the cycle, mimicking physiological ovarian stimulation.[232] Alternatively, pulsatile GnRH at a constant frequency also induces maturation of ovarian follicles and triggers an LH peak. The usual doses comprise 3–10 μg per pulse, injected subcutaneously every 90 min. Monitoring ovulation induction with GnRH is useful, as the risk of multiple pregnancy and ovarian hyperstimulation syndrome is much lower than that with gonadotropin therapy.[233] Once ovulation is obtained, the corpus luteum is stimulated to produce progesterone, which is necessary for embryo implantation. Short-term GnRH treatment is necessary to induce progesterone release until the endogenous secretion of hCG from the embryo begins.

Reducing long-term health effects of CHH

With appropriate and long-term treatment, many of the long-term effects of hypogonadism can be minimized. Adherence to treatment is key to supporting pubertal development, sexual function and psychological health.[234] Patients with psychosexual problems, low self-esteem and distorted body image might require psychological counselling and often benefit from peer–peer support.[98,192] Bone-health concerns in patients with CHH are well-recognized.[235] Lack of sex steroids is the presumed cause

of osteoporosis in patients with CHH[236–238] and although sex steroid treatment typically improves bone density, it does not fully reverse the phenotype. Notably, mutations in *FGF8*, *FGFR1* or *SEMA3A* might not only cause GnRH deficiency but also directly affect bone.[153,239] We recommend a baseline bone density measurement in all patients with CHH at final height and after 2 years of treatment. To date, insufficient evidence exists to use the WHO–FRAX risk calculator[240] in this population of patients. Long-term effects of hypogonadism also include an increased risk of developing metabolic problems. Several meta-analyses have demonstrated an association between low testosterone levels and the metabolic syndrome.[241–244] In male individuals, low testosterone levels are also strongly associated with an increased risk of developing type 2 diabetes mellitus.[245] However, few studies have examined metabolic risk specifically in patients with CHH. Interestingly, even short-term hypogonadism (such as that resulting from discontinuation of sex steroids for 2 weeks) induces increased fasting insulin concentrations in young men with CHH,[246] which suggests that adherence to treatment is also important for promoting metabolic health. Accordingly, therapeutic education is important for promoting adherence in the ongoing management of CHH.

Transitional care for CHH

Transition of young adults from paediatric care to adult care is a well-recognized challenge for patients with chronic endocrine conditions[247,248] including CHH.[175] For patients with CHH, discontinuation of care and resulting gaps in treatment can have considerable health consequences. Several types of model for transition exist from a simple transfer of care to more structured programmes.[249] Consensus among paediatric and adult endocrinologists in the European consortium studying GnRH biology (COST Action BM1105, http://www.gnrhnetwork.eu/) is that a structured transition is preferable and the process should include an evaluation of patient readiness, communication between providers and follow-up to ensure that the initial consultation has been completed. The timing of transition not only depends on structural aspects of a given health-care system but also on the individual patient's psychological and emotional development and capacity for autonomy and self-care.

Conclusions

CHH is a condition that is clinically and genetically heterogeneous. It is often challenging to diagnose, particularly during adolescence. In male individuals, cryptorchidism with or without micropenis might suggest neonatal CHH; however, similarly useful signs are lacking for female individuals. During adolescence, the diagnosis of CHH is difficult to discern from constitutional delay of growth and puberty. The presence of anosmia and/or olfactory bulb hypoplasia and/or aplasia (visualized by MRI) points to Kallmann syndrome, yet measurement of levels of serum gonadotropins, sex steroids, AMH and inhibin B are not always fully informative in confirming the diagnosis. At this stage, pubertal induction is commenced and a clinical and/or biochemical reassessment is conducted following therapeutic pause at final height, thus enabling a definitive diagnosis of CHH. Effective treatment is available not only for inducing virilization or estrogenization, but also for successful development of fertility. Advances in our understanding of the molecular basis of CHH have helped explain the genetic cause in 50% of cases and uncovered a complex model of genetics (oligogenicity) that might apply to a large proportion of patients. Furthermore, cases of reversal in adulthood point to gene–environment interactions.[6,7] To advance the field, identification of novel biomarkers to aid early diagnosis of CHH, assembly of a large CHH population to study the complex molecular basis of the disease, and development of targeted treatments to optimize fertility outcomes are important. The COST network was created to address these issues as well as to promote translational research into human reproduction and improve the management of CHH.

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