



# Genetics of congenital hypogonadotropic hypogonadism: peculiarities and phenotype of an oligogenic disease

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## Abstract

A genetic basis of congenital isolated hypogonadotropic hypogonadism (CHH) can be defined in almost 50% of cases, albeit not necessarily the complete genetic basis. Next-generation sequencing (NGS) techniques have led to the discovery of a great number of loci, each of which has illuminated our understanding of human gonadotropin-releasing hormone (GnRH) neurons, either in respect of their embryonic development or their neuroendocrine regulation as the “pilot light” of human reproduction. However, because each new gene linked to CHH only seems to underpin another small percentage of total patient cases, we are still far from achieving a comprehensive understanding of the genetic basis of CHH. Patients have generally not benefited from advances in genetics in respect of novel therapies. In most cases, even genetic counselling is limited by issues of apparent variability in expressivity and penetrance that are likely underpinned by oligogenicity in respect of known and unknown genes. Robust genotype–phenotype relationships can generally only be established for individuals who are homozygous, hemizygous or compound heterozygotes for the same gene of variant alleles that are predicted to be deleterious. While certain genes are purely associated with normosmic CHH (nCHH) some purely with the anosmic form (Kallmann syndrome—KS), other genes can be associated with both nCHH and KS—sometimes even within the same kindred. Even though the anticipated genetic overlap between CHH and constitutional delay in growth and puberty (CDGP) has not materialised, previously unanticipated genetic relationships have emerged, comprising conditions of combined (or multiple) pituitary hormone deficiency (CPHD), hypothalamic amenorrhea (HA) and CHARGE syndrome. In this review, we report the current evidence in relation to phenotype and genetic peculiarities regarding 60 genes whose loss-of-function variants can disrupt the central regulation of reproduction at many levels: impairing GnRH neurons migration, differentiation or activation; disrupting neuroendocrine control of GnRH secretion; preventing GnRH neuron migration or function and/or gonadotropin secretion and action.

## Introduction

Congenital hypogonadotropic hypogonadism (CHH) is characterized by deficient secretion or action of gonadotropin-releasing hormone (GnRH)—in the absence of any other pituitary hormone deficiencies, or structural parasellar

lesions—resulting in failure of gonadal hormone secretion and gametogenesis. By contrast, the majority of acquired diseases affecting the pituitary or hypothalamus—whether invasive, infiltrative, ischemic, traumatic, irradiational, inflammatory or metabolic—result in multiple hormone deficits. Once defined as “idiopathic”, a genetic cause is now apparent in almost 50% of CHH cases (Boehm et al. 2015; Young et al. 2019).

In respect of hypogonadotropic hypogonadism (HH) occurring as part of congenital combined pituitary hormone deficiency (CPHD), with or without non-endocrine anomalies, the aetiology is much less well understood and our knowledge is evolving more slowly, with over 90% of cases having no known genetic basis. However, alongside the classical CPHD genes encoding transcription factors central to embryonic pituitary cell fate specification, several CHH-associated genes have recently been found to underpin

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CPHD-associated HH (Raivio et al. 2012; Jayakody et al. 2012; McCabe et al. 2013; Izumi et al. 2014; McCormack et al. 2017).

Loss-of-function variants in CHH-associated genes can disrupt the central neuroendocrine regulation of reproduction at key points of vulnerability, comprising disrupted migration; differentiation or activation of GnRH neurons; disrupted neuroendocrine control of GnRH secretion; resistance to GnRH action; and primary impairment of gonadotropin secretion or action. In this review, we will examine the genetic basis of CHH and the lessons contained therein relating to human reproductive physiology and developmental biology.

## Epidemiology

Although widely considered to be a rare disease, ascertainment of its true prevalence is constrained by the scarcity of published literature. An historic study of Frenchmen called up for military service gave a male CHH prevalence of 1 in 4415 (Fromantin et al. 1973), but more recently, a Finnish retrospective study of hospital records gave a lower prevalence (Laitinen et al. 2011). While considered to be a male-predominant condition, with a reported male: female gender ratio of 3:6 in a study based on patients attending specialist centres (Dzemaili et al. 2017), this is not supported by our current understanding of the genetics implicated, wherein only 3.5–10% (according to the population) of unrelated CHH males harbour *ANOS1* mutations characteristic of X-linked inheritance (Sykiotis et al. 2010; Laitinen et al. 2011; Hanchate et al. 2012; Basaran et al. 2013; Tommiska et al. 2014; Stamou et al. 2019). The apparent male excess may reflect ascertainment bias, due to a greater likelihood of females being managed empirically by office- or community-based gynaecologists or primary care physicians. Indeed, closer examination of CHH kindreds (excluding those with *ANOS1* mutations) has found a sex ratio among affected individuals within kindreds that approaches parity (Francou et al. 2016; Maione et al. 2018).

Notably, significant phenotypic and genetic overlap between CHH and combined pituitary hormone deficiency (CPHD—resulting from dysregulated morphogenesis of pituitary gland) has been observed. CPHD has an estimated prevalence of around 1 in 8000 (Fang et al. 2016b), although the full spectrum of pituitary hormonal deficiencies may evolve over a variable duration of up to decades, not necessarily being able to manifest in its entirety at birth or in early childhood.

## Phenotypes

### CHH diagnosis and onset

CHH is defined clinically by the failure to initiate or complete normal puberty, with a variable defect in the maturation of secondary sexual characteristics, external genitalia, gametogenesis (Young et al. 2019) Table 1. Issues of sexual function and psychosocial adjustment are common in later life, possibly reflecting the consequences of delayed diagnosis and treatment (Dwyer et al. 2019b). The diagnosis of CHH is necessarily one of exclusion, since it requires acquired, functional or structural conditions to be ruled out, principally constitutional delay of growth and puberty (CDGP), parasellar lesions, pituitary iron overload, hyperprolactinemia, energy-deficit, metabolic-associated or drug-induced HH (Young et al. 2019). Having done so, CHH is clinically and biochemically manifested through low (or inappropriately normal) levels of gonadotropins, associated with amenorrhea and low oestradiol in females and low levels of total testosterone (TTe) in males (Boehm et al. 2015).

Nevertheless, in an otherwise fit and healthy young adult with preserved linear growth, there is little else other than CHH to account for the profile of gonadotropin-deficient absent puberty, particularly if clinical red flags for CHH are present, such as characteristic non-reproductive defects (i.e., anosmia, clefting, deafness), or features of absent neonatal minipuberty (history of cryptorchidism or micropenis) (Swee and Quinton 2019a). Although it might have been anticipated that CHH and CDGP would share elements of a

**Table 1** Signs and symptoms of isolated hypogonadotropic hypogonadism according to the onset of the disease

Age of onset	Males	Females
Foetal/neonatal	Microphallus Mild hypospadias Cryptorchidism	No phenotype
Minipuberty hormone profile	Low LH, FSH and testosterone	Low LH, FSH and AMH
Peri-pubertal	Absent or incomplete puberty Eunuchoid proportions Under-developed genitalia	Absent or incomplete puberty (Tanner B < 4) Eunuchoid proportions Primary amenorrhea
Adult	Erectile dysfunction Low libido Infertility Osteoporosis Depression/asthenia Anaemia	Secondary amenorrhea Infertility Osteoporosis

common genetic basis, this does not in fact appear to be the case (Cassatella et al. 2018).

TTe levels at diagnosis in males are typically very low ( $< 3.5$  nmol/L) (Pitteloud et al. 2002; Miraoui et al. 2013; Boehm et al. 2015), although somewhat higher levels have been reported in CHH males harbouring functionally validated homozygous mutations such as *GNRHR* Gln106Arg (de Roux et al. 1997; Costa et al. 2001; Pitteloud et al. 2001; Cangiano et al. 2019). According to whether puberty is arrested after a normal initiation, with some growth of testicular volume and activation of the pituitary–gonadal axis, or never started, it is possible to distinguish partial and complete forms of CHH (Fig. 1).

This distinction is important because partial activation of the HPG axis at puberty suggests a milder underlying defect, with the likelihood that gonadotropin and gonadal steroid secretion during perinatal minipuberty (lasting from 2 months antepartum to 3–6 months after birth) was at least partly conserved. CHH males with partial or arrested puberty at presentation (e.g., testis volume  $> 4$  mL) exhibit low-amplitude, low-frequency, or sleep-entrained-only LH pulses and have significantly better fertility outcomes with gonadotropin treatment. However, the majority (around 2/3) of CHH patients present with absent puberty (e.g., testis volume  $\leq 4$  mL) and show a flat LH profile on frequent sampling (Pitteloud et al. 2002) (Fig. 1).

In 1997 Nachtigall et al. described a form of idiopathic acquired isolated HH in adulthood (AHH), with similar hypotestosteronaemia (TTe  $< 4$ – $4.5$  nmol/L), with all possible secondary causes rigorously excluded (Nachtigall et al. 1997; Dwyer et al. 2010). Manifestations of AHH include infertility, sexual dysfunction, asthenia and reduced body hair, but with no clinical features to suggest prior incomplete pubertal maturation, with the potential for transmission of

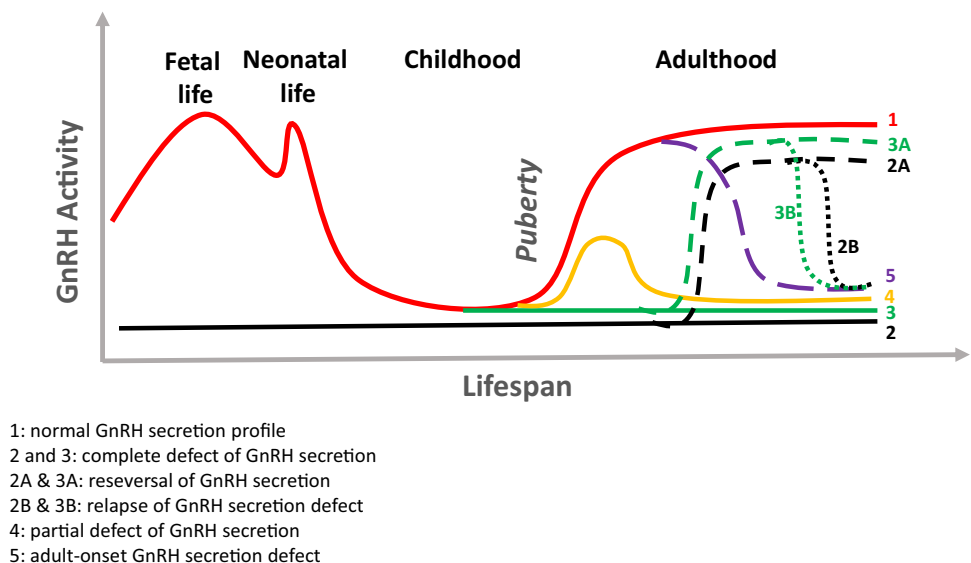
genes to the offspring prior to the onset of infertility. This could explain the existence of CHH families (Nachtigall et al. 1997; Dwyer et al. 2010) (Table 1).

While the genetic basis of AHH is not well characterised (Stamou and Georgopoulos 2018), there is an enrichment of rare CHH genetic variants compared to the general population, especially in AHH subjects with TTe level below 6 nmol/L and onset before 41 years of age (Cangiano et al. 2019). AHH has also been associated with minor pubertal delay in earlier life, suggesting the possibility of an underlying pre-existing mild impairment of hypothalamic–pituitary–gonadal (HPG) axis (Gianetti et al. 2010; Dwyer et al. 2010; Zhu et al. 2015). Crucially, some AHH patients also harbour non-reproductive defects commonly associated with classical CHH of pre-pubertal onset, including anomalous olfactory bulbs and sulci on MRI (Bonomi et al. 2018), thus further supporting the idea of a shared genetic basis.

## Reversal

Conversely, CHH patients can revert—permanently or transiently—to a normal autonomous function of the reproductive axis (Quinton et al. 1999; Raivio et al. 2007; Duan et al. 2014) (Fig. 1). This phenomenon, known as reversal, is not uncommon, with a prevalence among the patients ranging from 5–10% (Quinton et al. 1999; Raivio et al. 2007; Duan et al. 2014) to 20% (Sidhoum et al. 2014). Even though mutations in *TAC3* and *TACR3* genes may particularly predispose to reversal (Gianetti et al. 2010; Root 2010), there is no reliable genotype–phenotype correlation and reversal has been reported also in patients harbouring deleterious variants in *PROKR2* (Sinisi et al. 2008); *FGFR1* (Raivio et al. 2007; Costa-Barbosa et al. 2013), *GNRHR* (Raivio

**Fig. 1** GnRH function during lifespan and different presentations of GnRH deficiency in congenital hypogonadotropic hypogonadism (Adaptation from (Palmert and Boepple 2001); Copyright permission granted from Oxford University Press; License n. 477821043851)



et al. 2007), *HS6ST1* (Costa-Barbosa et al. 2013), *SOX10* (Maione et al. 2016) and even *ANOS1* (Ribeiro et al. 2007).

Whilst some groups have found larger testicular volume and higher stimulated LH levels at diagnosis correlate with a higher possibility of reversal (Duan et al. 2014), others have not (Raivio et al. 2007); nonetheless an enlargement of testicular size during testosterone treatment or unexpected pregnancy in female partner should prompt brief discontinuation of the treatment followed by biochemical re-evaluation, to assess a potential recovery of an autonomous gonadal function. Crucially, reversal may not be sustained, indicating a persistent fragility or vulnerability of the HPG axis (Santhakumar et al. 2014), so that lifelong monitoring is recommended (Sidhoum et al. 2014).

No convincing cases of reversal have been documented in females, possibly because this is clinically so difficult to unravel from the scenario of energy-deficit hypothalamic amenorrhea, which is far more common and wherein periods of remission and relapse over time are characteristic.

### Olfactory defect

Although a distinct population arises from the neural crest, the majority of hypothalamic GnRH neurons originate in the olfactory placode around the 5th gestational week and migrate alongside the olfactory, vomeronasal and terminal nerves until they reach their final destination in the mediobasal hypothalamus, infundibulum and periventricular region (Chung and Tsai 2010; Casoni et al. 2016; Haines et al. 2018). This shared origin is the reason why CHH is associated with olfactory impairment in around 50% of cases, thereby defining Kallmann syndrome (Boehm et al. 2015; Young et al. 2019).

Once considered as entirely separate diseases, KS and nCHH may also constitute separate manifestations of the same genetic disease, since they often coexist in the same kindreds (Trarbach et al. 2007; Dodé and Hardelin 2009; Pitteloud et al. 2010; Brioude et al. 2010). Indeed, they partly share the same genetic milieu.

With the probable exception of *ANOS1* mutations in hemizygoty, which are almost invariably associated to complete loss of olfaction, mutations of CHH genes involved in GnRH neuron migration/axon guidance and GnRH neuron fate specification or differentiation (neurodevelopmental genes), have been associated with a variable degree of olfactory defect, ranging from normosmia, hyposmia to complete anosmia, i.e., with either KS or nCHH phenotypes. However, mutations of CHH genes associated with impairment of GnRH neuron activation, GnRH secretion or GnRH action at the gonadotroph level only cause nCHH (Hudson et al. 1994; Lewkowitz-Shpuntoff et al. 2012).

### Genotype–phenotype correlations in relation to non-reproductive developmental abnormalities

In addition to olfactory deficit and neonatal reproductive features of impaired third trimester androgenization from absent minipuberty (micropenis and cryptorchidism), there are other developmental defects that disproportionately affect CHH patients, including hearing impairment, renal abnormalities, mirror movements (bimanual synkinesia), craniofacial clefting and abnormalities of eye movements, features that are significantly more prevalent among KS patients than nCHH (Quinton et al. 2001; Bhagavath et al. 2006; Bonomi et al. 2018) Table 2. Moreover, even though precise genotype–phenotype correlations may be lacking in published studies, both synkinesia and renal agenesis are almost entirely restricted to males (Bonomi et al. 2018) and are most commonly associated with *ANOS1* mutations (Quinton et al. 2001), whereas dental agenesis and midline facial defects (as well as some distinctive digital bone abnormalities such as polydactyly, syndactyly and camptodactyly) are more prevalent among subjects harbouring deleterious variants of *FGF8* and *FGFR1* (Costa-Barbosa et al. 2013).

Cryptorchidism and micropenis are significantly more common in men with complete—rather than partial CHH (Pitteloud et al. 2002; Bonomi et al. 2018) and, independent of this, are also more frequent in KS patients than nCHH

**Table 2** Prevalence of developmental abnormalities in nCHH and KS

	Bonomi et al. (2018)		Quinton et al. (2001)		Costa-Barbosa et al. (2013)
	nCHH (275)	KS (184)	nCHH (44–112)	KS (22–103)	KS (219)
Bimanual synkinesia (%)	2.5	10.9	0 (46)	31 (77)	19
Renal anomalies (%)	0.4	11.5	0 (26)	15 (61)	8
Orofacial cleft + dental agenesis (%)	9.8	14.9	> 5 (103)	> 4 (112)	14–20
Eye movement disorder (%)	nd	nd	4.5 (22)	27 (44)	nd
Hearing loss (%)	5.2	7	0 (46)	8 (77)	15

nCHH normosmic congenital hypogonadotropic hypogonadism, KS Kallmann syndrome, nd no data

(Quinton et al. 2001; Bhagavath et al. 2006; Bonomi et al. 2012, 2018).

The prevalence of hearing loss (ranges from 5 to 15% of total CHH patients) is higher in KS (Quinton et al. 2001; Bonomi et al. 2018) and is usually associated to mutations in *CHD7*, *SOX10*, *IL17RD*, *ANOS1*, *SOX10* (Costa-Barbosa et al. 2013; Pingault et al. 2013; Takagi et al. 2014; Vaaralahti et al. 2014; Wang et al. 2018) and *SOX2* (Kelberman et al. 2006; Steevens et al. 2017). Sensorineural hearing defects also form part of complex syndromes associated with neuroendocrine genes such as *LHX3* and *DMXL2* (Bonfig et al. 2011; Pozza et al. 2012) Table 3.

It should be recognized that there may not always be a clearly dividing line—rather, a progressive fade-in—between the classification of “CHH-with-non-reproductive-defects” and “CHH as part of a complex syndrome”. This is particularly the case in respect of CHH versus CHARGE syndrome with *CHD7* variants, although genetics may be helpful here, with inherited point mutation associated with CHH and large de novo deletions with CHARGE (Balasubramanian et al. 2014). Nevertheless, a pragmatic approach would be to consider whether CHH defines the major burden of disease experienced by the patient or whether the clinical burden of non-reproductive anomalies predominates in daily life.

## Genetics

Since the discovery of *ANOS1* (formerly *KALI*) in the pathogenesis of X-linked KS, many more genes have been evaluated in relation to CHH. The clinical relevance of variants in *ANOS1*, *FGFR1* and *GNRH/GNRHR* and *PROK2/PROKR2* has been confirmed in many studies, with more than fifty different variants in each gene, or pair of genes, described as “disease causing” in the Human Gene Mutation Database, HGMD (Stenson et al. 2014). However, for genes described more recently, there is wide variation in the strength, number and consistency of reported evidence, in the number of variants accepted as pathogenic, and in the percentage of total CHH cases estimated to be underpinned by disease-causing variants.

*KISS1/KISS1R* and *TAC3/TAC3R* are consolidated candidate loci, with proof of pathogenicity arising from different studies and having over 25 different verified disease-causing variants in relation to nCHH.

Other genes such as *SEMA3A*, *FGF8*, *SOX2*, *SOX10* are accumulating more evidence, having more than ten different disease-causing variants for CHH. *CHD7* is strongly associated both with CHARGE syndrome (in which CHH is embedded within a particularly severe combination of non-reproductive phenotypes) and also a significant proportion of nCHH and KS cases.

Much less common, but equally strong in terms of causation, are *LEP/LEPR* in the pathogenesis of CHH associated with childhood-onset hyperphagia and obesity, along with genes such as *DMXL2* and *SMCHD1* that cause specific syndromes with CHH as a part of a complex phenotype, and genes such as *OTUD4*, *RNF216*, *PNPLA6*, *STUB1*, *POLR3A* and *POLR3B* that cause neurologic syndromes combined with CHH.

Genes such as *NDNF*, *AMH/AMHR2*, *NTN1/DCC*, *FEZF1*, *HS6ST1* and *NMSF1* have only been functionally validated in very few or even single studies and so have not yet passed the test of reproducibility to confirm their role. Moreover, humans’ variants in *DUSP6*, *FLRT3*, *SPRY4*, *CCDC141*, *SEMA7A*, *TBX3* and *GLCE* entirely lack functional validation. Finally, the CPHD-associated genes account only for a minimal proportion of isolated CHH.

Another approach to understanding the physiological importance of each locus, and hence its role in the pathogenesis of CHH, is to examine the prevalence of rare genetic variants among patient cohorts compared to control populations, with significant enrichment of rare variants in these loci in CHH indicating a major disease-causing role (see Table 4). Hitherto, this approach has performed well whenever it has been possible to test against conventional gene validation techniques.

Herein, we report all genes linked with CHH—listed according to their prevalent pathogenic mechanism—to provide a quick and useful reference for clinicians and scientists seeking information on one or more specific genes. For each gene, we present available knowledge about pathophysiology, inheritance, associated phenotypes and the types of variants reported in literature. Many of the genes implicated in CHH are G-protein-coupled receptors (GPCRs) and their cognate ligands, with mutations of the receptor usually being an order of magnitude more frequent. Thus, where the phenotype is clearly similar, we have described the effects of receptor and ligand allelic variants in the same section.

With the possible exception of the most deleterious *FGFR1*, *TUBB3*, *SMCHD1* and *CHD7* mutations, it may be that none of the variants described below is able, of itself, to cause a CHH disease phenotype in the heterozygous state. Where a sufficient number of cases have been described having homozygosity (or compound heterozygosity) for a given gene, then a plausible genotype–phenotype correlation can be outlined with some certainty. However, in many cases, this is not possible and, in particular, the relative impacts of other known or unknown genetic variants may be hard to disentangle.

**Table 3** Genetics of hypogonadotropic hypogonadism

Gene name	Gene symbol	Locus	OMIM	Inheritance	Phe-notype MIM number	Main physio-pathological mechanism	Ofactory defect	Onset	Reversal	Ataxia	Other pituitary defects	Possible associated syndromic features	Hearing loss	Midline defects	Mirror movements	Renal agenesis/hyoplasia	Animal model	Human variants functionally validated
Leptin	LEP	7q32.1	164160	AR	614962	Energy-deficit HH	nCHH	CHH	Unknown			Obesity					Mouse	Yes
Leptin receptor	LEPR	1p31.3	601007	AR	614963	Energy-deficit HH	nCHH	CHH	Unknown			Obesity					Mouse	Yes
Gonadotropin-releasing Hormone 1	GnRH1	8p21.2	152760	AR olig	614841	GnRH function	nCHH	CHH	Unknown								Mouse	Yes
Gonadotropin-releasing Hormone receptor	GnRHR	4q13.2	138,850	AR olig	146110	GnRH function	nCHH	CHH or AHH	Yes								Mouse	Yes
Kiss1 metastasis suppressor	KISS1	1q32.1	603286	AR	614842	GnRH neuron activation	nCHH	CHH	Yes								Mouse	Yes
Kiss1 receptor	KISS1R	19p13.3	604161	AR	614837	GnRH neuron activation	nCHH	CHH	Yes								Mouse	Yes
Tachykinin 3	TAC3	12q13.3	162330	AR	614839	GnRH neuron activation	nCHH	CHH	Yes								Rat/mouse	Yes
Tachykinin receptor 3	TACR3	4q24	162332	AR olig	614840	GnRH neuron activation	nCHH	CHH	Yes								Rat/mouse	Yes
Anosmin 1	ANOS1 (KAL1)	Xp22.31	300836	XLR	308700	GnRH migration/axon guidance	KS	CHH	Yes				+		+	+	<i>C. elegans</i>	Yes
Heparan sulfate 6-O-sulfotransferase 1	HS6ST1	2q14.3	604846	Olig	614880	GnRH migration/axon guidance	KS or nCHH	CHH or AHH	Yes								<i>C. elegans</i>	Yes

**Table 3** (continued)

Gene name	Gene symbol	Locus	OMIM	Inheritance	Phe-notype MIM number	Main physio-pathological mechanism	Olfactory defect	Onset	Reversal	Ataxia	Other pituitary defects	Possible associated syndromic features	Hearing loss	Midline defects	Mirror movements	Renal agenesis/hyoplasia	Animal model	Human variants functionally validated
Prokineticin 2	PROKR2	3p13	607002	AR, AD, olig	610628	GnRH migration/axon guidance-ance	KS or nCHH	CHH	Unknown						+		Mouse	Yes
Prokineticin receptor 2	PROKR2	20p12.3	607123	AR, AD, olig	244200	GnRH migration/axon guidance-ance	KS or nCHH	CHH or AHH	Yes		GH, TSH, ACTH	SOD			+		Mouse	Yes
Semaphorin 3A	SEMA3A	7p12.1	603961	AD, olig	614897	GnRH migration/axon guidance-ance	KS or nCHH	CHH	Unknown								Mouse	Yes
Plexin A1	PLXNA1	3q21.3	601055	AR, olig	–	GnRH migration/axon guidance-ance	KS or nCHH	CHH	Unknown								Mouse	No
Semaphorin 7A	SEMA7A	15q24.1	607961	Olig	–	GnRH migration/axon guidance-ance	KS or nCHH	CHH	Unknown								Mouse	No
Semaphorin 3E	SEMA3E	7q21.11	608166	Olig	214800	GnRH migration/axon guidance-ance	KS or nCHH	CHH or AHH	Unknown			CHARGE					Mouse	Yes
NMDA Receptor synaptic-clearing and neuronal migration Factor	NSMF (NELF)	9q34.3	608137	AR, olig	614838	GnRH migration/axon guidance-ance	KS or nCHH	CHH	Unknown								Mouse	Yes

Table 3 (continued)

Gene name	Gene symbol	Locus	OMIM	Inheritance	Phe-notype MIM number	Main physiopathological mechanism	Olfactory defect	Onset	Reversal	Ataxia	Other pituitary defects	Possible associated syndromic features	Hearing loss	Midline defects	Mirror movements	Renal agenesis/hyoplasia	Animal model	Human variants functionally validated
Coiled-coil domain-containing protein 141	CCDC141	2q31.2	616031	AR, olig	–	GnRH migration/axon guidance	nCHH	CHH	Yes								Mouse	No
FEZ family zinc finger protein 1	FEZF1	7q31.32	613301	AR	616030	GnRH migration/axon guidance	KS	CHH	Unknown								Mouse	Yes
DCC Netrin 1 receptor	DCC	18q21.2	120470	AD, olig	–	GnRH migration/axon guidance	KS or nCHH	CHH	Unknown					+	+		Mouse	Yes
Netrin 1	NTN1	17p13.1	601614	AD, olig	–	GnRH migration/axon guidance	KS or nCHH	CHH	Unknown					+	+		Mouse	Yes
Anti-Müllerian Hormone	AMH	19p13.3	600957	AD	–	GnRH migration/axon guidance	KS or nCHH	CHH	Unknown								Mouse	Yes
Anti-Müllerian Hormone type II receptor	AMHR2	12q13.13	600956	AD	–	GnRH migration/axon guidance	KS or nCHH	CHH	Unknown								Mouse	Yes
Neuron-Derived Neurotrophic Factor	NDNF	4q27	616506	AD	–	GnRH migration/axon guidance	KS	CHH	Unknown								Mouse/zebrafish	Yes



**Table 3** (continued)

Gene name	Gene symbol	Locus	OMIM	Inheritance	Phe-notype MIM number	Main physi-opathological mechanism	Olfactory defect	Onset	Reversal	Ataxia	Other pituitary defects	Possible associated syndromic features	Hear-ing loss	Midline defects	Mirror move-ments	Renal agen-esis/hy-po-plasia	Animal model	Human variants func-tionally validated
Sry-box 10	SOX10	22q13.1	602229	AD	611584	GnRH migra-tion/axon guid-ance	KS	CHH	Yes			Waarden-burg	+				Mouse	Yes
Tubulin, beta-3	TUBB3	16q24.3	602661	AD	–	GnRH migra-tion/axon guid-ance	KS	CHH	Unknown			Moebius		+			Mouse	Yes
Gluconic acid epime-rase	GLCE	15q23	612134	–	–	GnRH migra-tion/axon guid-ance	KS or nCHH	CHH	Unknown								Mouse	No
Fibroblast growth factor receptor 1	FGFR1	8p11.23	136350	AD, AR, olig	147950	GnRH neuron specifi-cation	KS or nCHH	CHH	Yes		GH, TSH, ACTH	Hartsfield		+	+		Mouse	Yes
Interleukin 17 receptor D	IL17RD	3p14.3	606807	Olig	615267	GnRH neuron specifi-cation	KS or nCHH	CHH	Unknown				+				Mouse	Yes
Fibroblast growth fac-tor 17	FGF17	8p21.3	603725	Olig	615270	GnRH neuron specifi-cation	KS or nCHH	CHH	Unknown								Mouse	Yes
Fibroblast growth fac-tor 8	FGF8	10q24.32	600483	Olig	612702	GnRH neuron specifi-cation	KS or nCHH	CHH or AHH	Yes		GH, PRL, TSH, ACTH	SOD		+	+		Mouse	Yes
Dual-speci-ficity phos-phatase 6	DUSP6	12q21.33	602748	Olig	615269	GnRH neuron specifi-cation	KS or nCHH	CHH	Unknown				+				Mouse	No

Table 3 (continued)

Gene name	Gene symbol	Locus	OMIM	Inheritance	Phe-notype MIM number	Main physiopathological mechanism	Olfactory defect	Onset	Reversal	Ataxia	Other pituitary defects	Possible associated syndromic features	Hearing loss	Midline defects	Mirror movements	Renal agenesis/hyoplasia	Animal model	Human variants functionally validated
Fibronectin-like domain-containing leucine-rich transmembrane protein 3	FLRT3	20p12.1	604808	Olig	615271	GnRH neuron specification	KS or nCHH	CHH	Unknown					+			Mouse	No
Sprouty, drosophila, homolog of, 4	SPRY4	5q31.3	607984	Olig	615266	GnRH neuron specification	KS or nCHH	CHH or AHH	Unknown								Mouse	No
Beta-Klotho	KLB	4p14	611135	AD	–	GnRH neuron specification	KS or nCHH	CHH	Yes					+		+	Mouse	Yes
WD repeat-containing protein 11	WDR11	10q26.12	606417	AD, olig	614858	GnRH neuron specification	KS or nCHH	CHH	Unknown		GH, TSH		+				Mouse	Yes
Immunoglobulin superfamily, member 10	IGSF10	3q25.1	617351	AD	–	GnRH neuron specification	nCHH	CHH	Unknown								Mouse, zebrafish	Yes
Nuclear receptor subfamily 0, group B, member 1	NR0B1 (DAX1)	Xp21.2	300473	XLR	300200	GnRH neuron specification	nCHH	CHH	Unknown			CAH					Mouse	Yes
Chromodomain helicase DNA-binding protein 7	CHD7	8q12.2	608892	AD, AR, olig	612370	GnRH neuron specification	KS or nCHH	CHH	Yes		GH, TSH	CHARGE	+				Mouse	Yes
Sry-box 2	SOX2	3q26.33	184429	AR	206900	GnRH neuron specification	nCHH	CHH	Unknown		GH, TSH	SOD	+				Mouse	Yes
Pituitary (Classically CPHD)																		
Lim homeobox gene 4	LHX4	1q25.2	602146	AD	262700	Gonadotroph specification	nCHH	CHH	Unknown		GH, PRL, TSH, ACTH						Mouse	Yes

**Table 3** (continued)

Gene name	Gene symbol	Locus	OMIM	Inheritance	Phe-notype MIM number	Main physiopathological mechanism	Ofactory defect	Onset	Reversal	Ataxia	Other pituitary defects	Possible associated syndromic features	Hearing loss	Midline defects	Mirror movements	Renal agenesis/hypoplasia	Animal model	Human variants functionally validated
Homeobox gene expressed in ES cells	HESX1	3p14.3	601802	AD, AR	182230	Gonadotroph specification	KS or nCHH	CHH	Unknown		GH, PRL, TSH, ACTH, ADH	SOD					Mouse	Yes
Lim Homeobox gene 3	LHX3	9q34.3	600577	AR	221750	Gonadotroph specification	nCHH	CHH	Unknown		GH, PRL, TSH, ACTH		+				Mouse	Yes
SRY-box 3	SOX3	Xq27.1	313430	XLR	312000	Gonadotroph specification	nCHH	CHH	Unknown		GH, TSH, ACTH	SOD	+				Mouse	Yes
Orthodenticle, drosophila, homolog Of, 2	OTX2	14q22.3	600037	AD	613986	Gonadotroph specification	nCHH	CHH	Unknown		GH, PRL, TSH, ACTH	SOD					Mouse	Yes
Prop Paired-Like Homeobox 1	PROP1	5q35.3	601538	AR	262600	Gonadotroph specification	nCHH	CHH	Unknown		GH, PRL, TSH, ACTH						Mouse	Yes
Gli-Kruppel family member 2	GLI2	2q14.2	165230	AD	615849	Gonadotroph specification	nCHH	CHH	Unknown		GH, PRL, TSH, ACTH, ADH	Culler-Jones		+			Mouse	Yes
Paired-like homeodomain transcription factor 2	PITX2	4q25	601542	AD	180500	Gonadotroph specification	nCHH	CHH	Unknown		GH, PRL, TSH	Axenfeld-Rieger					Mouse	No
Gata-binding protein 2	GATA2	3q21.3	137295	AD	-	Gonadotroph specification	nCHH	CHH	Unknown		TSH						Mouse	No
Proprotein convertase, subtilisin/kexin-type, 1	PCSK1	5q15	162150	AR	600955	Gonadotrophin secretion or action	nCHH	CHH	Unknown		ACTH	Obesity					Mouse	Yes

Table 3 (continued)

Gene name	Gene symbol	Locus	OMIM	Inheritance	Phe-notype MIM number	Main physiopathological mechanism	Olfactory defect	Onset	Reversal	Ataxia	Other pituitary defects	Possible associated syndromic features	Hearing loss	Midline defects	Mirror movements	Renal agenesis/hyoplasia	Animal model	Human variants functionally validated
Luteinizing Hormone, beta polypeptide	LHB	19q13.33	152780	AR	228300	Gonadotropin secretion or action	nCHH	CHH	Unknown		isolated LH						Mouse	Yes
Follicle-Stimulating Hormone, beta polypeptide	FSHB	11p14.1	136530	AR	229070	Gonadotropin secretion or action	nCHH	CHH	Unknown		isolated FSH						Mouse	Yes
Syndromic DMX-like 2	DMXL2	15q21.2	612186	AD	616113	GnRH neuron activation	nCHH	CHH	Unknown		TSH	Polyendocrine-ropathy	+				Mouse	Yes
Structural maintenance of chromosome flex-ible hinge domain-containing protein 1	SMCHD1	18p11.32	614982	AD	603457	GnRH migration/axon guidance	KS	CHH	Unknown			Bosma arhinia-microphthalmia					Zebrafish	Yes
T-box 3	TBX3	12q24.21	601621	AD	181450	Gonadotropin specification	nCHH	CHH	Unknown		GH	Ulnar-mammary		+			Mouse	No
OTU domain-containing protein 4	OTUD4	4q31.21	611744	AR, olig	212840	Gonadotropin secretion or action	nCHH	CHH	Unknown	+		Gordon Holmes					Zebrafish	Yes
Ring finger protein 216	RNF216	7p22.1	609948	AR, olig	212840	Gonadotropin secretion or action	nCHH	CHH	Unknown	+		Gordon Holmes					Zebrafish	Yes

**Table 3** (continued)

Gene name	Gene symbol	Locus	OMIM	Inheritance	Phe-notype MIM number	Main physi-ological mecha-nism	Olfactory defect	Onset	Reversal	Ataxia	Other pituitary defects	Possible associated syndromic features	Hear-ing loss	Midline defects	Mirror move-ments	Renal agen-esis/hypo-plasia	Animal model	Human variants func-tionally validated
Patatin-like phospholipase domain-containing protein 6	PNPLA6	19p13.2	603197	AR	215470	Gonadotropin secretion or action	nCHH	CHH	Unknown	+		Gordon Holmes, Boucher-Neuhäuser					Drosophila	Yes
Stip1 homologous and U box-containing protein 1	STUB1	16p13.3	607207	AR	615768	Gonadotropin secretion or action	nCHH	CHH	Unknown	+		Gordon Holmes					Mouse	Yes
Polymerase III, RNA, subunit A	POLR3A	10q22.3	614258	AR	607694	Gonadotropin secretion or action	nCHH	CHH	Unknown	+		Hypomye-leukodyst, Wiede-mann-Rauten-strauch					Mouse	Yes
Polymerase III, RNA, subunit B	POLR3B	12q23.3	614366	AR	614381	Gonadotropin secretion or action	nCHH	CHH	Unknown	+		Hypomye-leukodyst, 4H					Mouse	Yes

*SOD* septo-optic dysplasia, *olig*, oligogenic, *hypomy leukodyst* hypomyelinating leukodystrophy, *CAH* congenital adrenal hypoplasia

**Table 4** Prevalence of rare variants in candidate genes in CHH cohorts

Gene	% (proportion)	References
<i>FGFR1</i>	8.9% (100/1137)	Sykiotis et al. (2010), Laitinen et al. (2011), Hanchate et al. (2012), Tommiska et al. (2014), Wang et al. (2017), Men et al. (2019)
<i>CHD7</i>	8.1% (48/592)	Kim et al. (2008a), Hanchate et al. (2012), Wang et al. (2017), Gonçalves et al. (2019), Stamou et al. (2019)
<i>PROKR2</i>	5.6% (87/1547)	Cole et al. (2008), Abreu et al. (2008), Sykiotis et al. (2010), Hanchate et al. (2012), Libri et al. (2014), Stamou et al. (2019)
<i>SEMA3A</i>	5.3% (28/523)	Hanchate et al. (2012), Käsäkoski et al. (2014), Stamou et al. (2019)
<i>ANOS1</i>	4.5% (46/1027)	Sykiotis et al. (2010), Laitinen et al. (2011), Hanchate et al. (2012), Basaran et al. (2013), Tommiska et al. (2014), Stamou et al. (2019)
<i>GNRHR</i>	4.4% (55/1245)	Sykiotis et al. (2010), Basaran et al. (2013), Tommiska et al. (2014), Francou et al. (2016), Gonçalves et al. (2017), Stamou et al. (2019)
<i>SOX10</i>	4.4% (11/248)	Pingault et al. (2013), Dai et al. (2019)
<i>SEMA7A</i>	4% (2/50)	Käsäkoski et al. (2014)
<i>KLB</i>	4% (13/344)	Xu et al. (2017)
<i>SPRY4</i>	3.2% (15/473)	Miraoui et al. (2013), Stamou et al. (2019)
<i>PROK2</i>	2.4% (33/1347)	Cole et al. (2008), Abreu et al. (2008), Sykiotis et al. (2010), Hanchate et al. (2012), Basaran et al. (2013), Wang et al. (2017)
<i>SOX2</i>	2.3% (2/87)	Stamou et al. (2019)
<i>DMXL2</i>	2.3% (2/87)	Stamou et al. (2019)
<i>IL17RD</i>	2.1% (8/386)	Miraoui et al. (2013)
<i>KISS1R</i>	1.6% (18/1087)	Sykiotis et al. (2010), Francou et al. (2016), Stamou et al. (2019)
<i>GNRH1</i>	1.5% (12/770)	Chan et al. (2009), Francou et al. (2016)
<i>TACR3</i>	1.5% (10/651)	Francou et al. (2011, 2016), Stamou et al. (2019)
<i>HESX1</i>	1.4% (3/217)	Newbern et al. (2013)
<i>DUSP6</i>	1.3% (5/386)	Miraoui et al. (2013)
<i>FGF8</i>	1.2% (11/928)	Sykiotis et al. (2010), Hanchate et al. (2012), Men et al. (2019)
<i>FGF17</i>	1.1% (6/531)	Miraoui et al. (2013), Men et al. (2019)
<i>POLR3A</i>	1.1% (1/87)	Stamou et al. (2019)
<i>POLR3B</i>	1.1% (1/87)	Stamou et al. (2019)
<i>PNPLA6</i>	1.1% (1/87)	Stamou et al. (2019)
<i>NSMF</i>	1.0% (of 397)	Sykiotis et al. (2010)
<i>TAC3</i>	0.9% (5/564)	Francou et al. (2011)
<i>FLRT3</i>	0.8% (3/386)	Miraoui et al. (2013)
<i>KISS1</i>	0% (0/307)	Francou et al. (2016)

## Genes involved in GnRH neuron migration/axon guidance

### *ANOS1* (formerly *KAL1*)

*ANOS1* encodes for an *N*-glycosylated protein called Anosmin-1, which is both expressed on the cell surface (Soussi-Yanicostas et al. 1996) and secreted (Rugarli et al. 1996). In 1991, it was the very first causal gene identified for CHH, discovered through positional cloning of DNA from patients having contiguous genes syndrome at Xp22.3 (Franco et al. 1991; Legouis et al. 1991). It is also the only gene in which a gene-deleted human foetus has been studied scientifically, showing failure of olfactory bulb development, with olfactory, terminal and vomeronasal axons ending in neurofibrillary tangles at the cribriform plate and GnRH neurons

accumulating in the upper nasal septum and cribriform plate areas (Schwanzel-Fukuda et al. 1989).

The mode of inheritance is characteristically X-linked recessive, from the maternal healthy carrier to hemizygous affected male offspring. More than sixty mutations have been described until now, most of them nonsense mutations or large exon deletions. Nonetheless, a small number of missense variants have also been described in reported KS cases, principally affecting the whey acidic protein-like or the fibronectin-like type 3 domains and, hence, responsible for an impairment of disulphide bond formation and heparan binding, respectively (Kim et al. 2008b).

*ANOS1* mutations have a high penetrance and expressivity and they are usually associated with a severe olfactory and reproductive phenotype (Oliveira et al. 2001; Salenave et al. 2008; Cangiano et al. 2019), with only one case of

nCHH hitherto reported (Sato et al. 2004). Mutations in *ANOS1* are also associated with bimanual synkinesia, midline defects, hearing loss and renal agenesis, which might relate to the known expression of *ANOS1* in the developing kidney and other relevant sites (Duke et al. 1995; Costa-Barbosa et al. 2013).

The cited prevalence of *ANOS1* mutations among affected individuals varies according to whether the study cohort comprised both sexes versus just males, total CHH versus just KS individuals, according to the proportion of sporadic cases versus familial cases and whether only unrelated individuals were included. These variables are not always clear from the literature. However, the prevalence of *ANOS1* mutations among unrelated sporadic KS males may vary between 3.5 and 10% (Sykiotis et al. 2010; Laitinen et al. 2011; Hanchate et al. 2012; Basaran et al. 2013; Tommiska et al. 2014; Stamou et al. 2019).

Anosmin-1, the encoded glycoprotein, belongs to the superfamily of adhesion molecule that are important for neuronal adhesion and axonal extension and guidance. Indeed, Anosmin-1 is fundamental to olfactory axon guidance since—once the soluble form is incorporated to the extracellular matrix of the olfactory bulb—it promotes cell migration and the final targeting of olfactory axons, acting as a chemoattractant as well as a branch-promoting factor.

Immortalized migratory GnRH neurons exhibit a cell-specific chemotactic reaction when exposed to Anosmin-1-enriched media (Cariboni et al. 2004); anti-Anosmin-1 antibodies block the correct development of rats' olfactory bulb branches in vitro (Soussi-Yanicostas et al. 2002) and, in *C. elegans*, an heterozygous mutation of the homologous of *ANOS1* caused a highly penetrant axon-misrouting phenotype (Bülow et al. 2002). Moreover, Anosmin-1 co-localizes with *FGFR1* in the olfactory structures during development (Ayari and Soussi-Yanicostas 2007) and it has been shown that *ANOS1* could enhance FGF signalling interacting with FGFR–FGF–heparan sulphate proteoglycan complex on the cell surface (Gonzalez-Martinez 2004). However, functional studies in animal models have been severely circumscribed by the absence of a murine orthologue (Vezzoli et al. 2016).

### ***HS6ST1***

*HS6ST1* encodes an enzyme that non-randomly introduces a sulphate in the 6-*O*-position of heparan sulphate—an extracellular matrix element important for guiding cell-to-cell communications during neural development and migration. Both in vivo and in vitro functional studies have shown that heparan sulphate proteoglycan chains are involved in the interaction and binding of Anosmin-1 to the cell membranes (Soussi-Yanicostas et al. 1996). Studies in *C. elegans* demonstrated that alteration of heparan 6-*O*-sulfotransferase worsened the *ANOS1*-related axonal defects (Bülow et al.

2002), suggesting that Anosmin-1 binds through this heparan sulphate proteoglycan to its cognate receptor (or other extracellular cues) to induce axonal branching and—if the system is altered—it can lead to axon misrouting. Moreover, heparan sulphate 6-*O*-sulfotransferase may also be involved in activation of the *FGFR1* pathway through its synergy with Anosmin-1 (Tornberg et al. 2011).

In humans, missense allelic variants affecting highly conserved residues of *HS6ST1* were first described in a cohort of 338 CHH patients (Tornberg et al. 2011) and a subsequent report found an association of *HS6ST1* variants with both reversal and oligogenicity (Costa-Barbosa et al. 2013). Along with the great variance in the expressivity of the gene variants described, these observations suggest that alterations of *HS6ST1* alone might not be sufficient to produce a CHH phenotype. Indeed, a recent report demonstrated the possible involvement of an *HS6ST1* variant in case of self-limited delayed puberty (Howard et al. 2018) in contrast with other GnRH deficiency gene (Cassatella et al. 2018).

### ***PROK2-PROKR2***

*PROK2*, and its GPCR, *PROKR2*, are primarily expressed in the arcuate nucleus, the olfactory tract and the supra-chiasmatic nucleus and are fundamental for migration and development of both GnRH and olfactory neurons (Ng 2005; Prosser et al. 2007). They were first described to be associated with CHH by Dodé et al. (2006), with *Prok2/Prokr2* mutations causing agenesis or hypoplasia of the olfactory bulbs and abnormal migration of GnRH neurons in mice (Matsumoto et al. 2006; Pitteloud et al. 2007b). Associated phenotypes in humans include fibrous dysplasia, synkinesia and epilepsy (Cole et al. 2008).

Since then, many variants have been found in human CHH patients, both with and without anosmia, and in vitro studies have demonstrated a deleterious effect on downstream signalling (Dodé et al. 2006; Pitteloud et al. 2007b; Leroy et al. 2008; Cole et al. 2008; Monnier et al. 2009; Abreu et al. 2012; Libri et al. 2014; Cox et al. 2018). More recently, it was demonstrated that *PROKR2* variants might also be responsible for a “biased signalling” effect on the receptor; indicating the need to test all potential couplings of *PROKR2* when a likely causal allelic variant protein is pharmacologically tested (Libri et al. 2014; Sbai et al. 2014).

The majority of *PROK2* and *PROKR2* mutations are missense variants and patients exhibit an inconsistent phenotype due to a variable expressivity and penetrance. Whilst homozygous mutations are associated with a severe phenotype of high penetrance (Dodé et al. 2006; Abreu et al. 2008; Sarfati et al. 2010; Libri et al. 2014), *PROKR2* heterozygous variants showed no genotype–phenotype correlation at all, in the pedigrees reported (Dodé et al. 2006; Falardeau et al. 2008; Monnier et al. 2009; Caronia et al. 2011; Raivio et al.

2012; Abreu et al. 2012; Sbai et al. 2014). Indeed, using criteria of the American College of Medical Genetics and Genomics, a recent reanalysis of published *PROKR2* allelic variants—hitherto considered to be pathogenic—reassigned several of them to the category of uncertain significant or even benign (Cox et al. 2018). However, a similar reanalysis of (much rarer) published *PROK2* variants is clearly overdue.

Moreover, certain functionally validated variants that were initially considered to have an autosomal dominant (AD) transmission with full expressivity and penetrance (Dodé et al. 2006), were subsequently also found in AHH (Libri et al. 2014; Cangiano et al. 2019), in cases of reversal (Sinisi et al. 2008) and in unaffected subjects (Pitteloud et al. 2007b; Abreu et al. 2008). Therefore, as the heterozygous phenotype is often expressed in associations with rare variants in other genes, oligogenicity may be an obligate requirement for heterozygous variants of *PROK2/PROKR2* to cause disease (Leroy et al. 2008; Sykiotis et al. 2010).

Due to the discordant results of functional studies, it remains uncertain whether certain variants can exert a dominant-negative effect. For instance, in evaluating the in vitro co-expression of a mutated and a wild-type allele, (Monnier et al. 2009) found both no impairment in the signal transmission or other evidence supporting a dominant-negative effect, allowing autosomal dominant transmission, whereas (Abreu et al. 2012) and (Cox et al. 2018) did so.

Finally, heterozygous *PROKR2* mutations—validated in vitro—are also associated with CPHD and septo-optic dysplasia (SOD), suggesting a role of this gene in pituitary development, which is supported by expression of *Prokr2* in the pars nervosa of the pituitary in the murine animal model (Raivio et al. 2012; McCormack et al. 2017).

### Semaphorin signalling (*SEMA3A*, *SEMA3E*, *SEMA7A*, *PLXNA1*)

Semaphorins belong to a family of proteins that—besides being crucial in the control of immune and vascular systems and tumorigenesis—play a pivotal role in neural circuit development, principally by controlling axon guidance processes. This guidance action is fundamental also for development of the olfactory system and the migration of GnRH neurons and many genes in this family have been linked to CHH (Messina and Giacobini 2013; Oleari et al. 2019b).

*SEMA3A* encodes a class 3 (i.e., interacting with neuropilins as co-receptor for the ligand) semaphorin that is expressed in the olfactory system as a secreted protein—which is essential for guidance of vomeronasal (Cariboni et al. 2011) and olfactory (Pasterkamp et al. 1998) axons—and was found to underpin human CHH in 2012 (Young et al. 2012; Hanchate et al. 2012). Data from a murine model demonstrated that *Sema3a* deletions, or missense mutations in the binding domain of *NRPI* (encoding

*SEMA3A*'s co-receptor, Neuropilin-1), impair GnRH neuron migration and normal development of the olfactory system (Cariboni et al. 2011; Hanchate et al. 2012).

*SEMA3A* variants in CHH patients are usually missense mutations found in a heterozygous state; once believed to be a gene with an autosomal dominant transmission, the complex pattern of inheritance together with accumulating evidence of associated variants in other CHH genes, now more convincingly signposts oligogenicity (Young et al. 2012; Hanchate et al. 2012; Käsäkoski et al. 2014).

Plexin-A1, encoded by *PLXNA1*, is the receptor activated by Semaphorin-3A and Neuropilin-1 and is important for neuronal development during embryonic growth. It is expressed in the olfactory system and the vomeronasal organ and nerve (Messina and Giacobini 2013; Marcos et al. 2017). Studies in the mouse have demonstrated that homozygous gene deletions produce a KS-like phenotype, albeit with incomplete penetrance (Marcos et al. 2017). Very recently the synergistic action of another member of the *PLXNA* family has been demonstrated. Thus, double *Plxna1* and *Plxna3* knockout mice phenocopy the olfactory and GnRH neuron defects observed in *Sema3a*-null mice (Oleari et al. 2019a).

A missense heterozygous mutation in *SEMA3E*, in association with a heterozygous *CHD7* variant, affecting two brothers with Kallmann's Syndrome was recently reported using sequencing techniques and computational modelling (Cariboni et al. 2015). Functional studies demonstrated that the Semaphorin 3E protein product is necessary for GnRH neuron survival upon entering the brain (Cariboni et al. 2015). Further, a de novo *SEMA3E* missense variant was reported in a patient with CHARGE syndrome, thereby reinforcing the relationship between semaphorins and *CHD7* (Lalani et al. 2004). An enrichment in rare heterozygous *SEMA3E* variants in AHH is also consistent with variable expressivity (Cangiano et al. 2019).

Besides *SEMA3A* and *SEMA3E*, other components of the semaphorin family are being studied for their roles in neuron migration and as potential candidates for the pathogenesis of human CHH. Among them, the *Sema7a* ortholog was found to be involved in GnRH neuron migration in the mouse and in vitro, being widely expressed both in the nasal placode and along the olfactory/vomeronasal axonal scaffold and having an important role in the regulation of cell motility (Messina et al. 2011), the protein binds to Plexin-C1 to decrease integrin-mediated cell attachment and spreading (Messina and Giacobini 2013). A recent study found several *SEMA7A* heterozygous missense variants in CHH patients, albeit also in association with other candidate genes (Käsäkoski et al. 2014). Given the complex roles of semaphorins in axon guidance, neuronal migration, as well as in neuronal plasticity, it will not be no surprise if additional members of



the semaphoring–plexin signalling pathways are found to be implicated in CHH (Oleari et al. 2019b).

### ***NSMF***

NMDA receptor, synaptonuclear signalling and neuronal migration factor (*NSMF*), also called “nasal embryonic LHRH factor” (*NELF*), is expressed during murine olfactory and GnRH neuron development (Kramer and Wray 2000, 2001), and the encoded Jacob protein was believed to be required for guidance of olfactory axonal projections and consequent GnRH migration (Xu et al. 2010). *NMSF* variants were subsequently reported in human CHH (Miura et al. 2004; Tornberg et al. 2011). However, recent in vivo findings in murine *Nsmf* knockouts do not support a major role for Jacob protein in the migration of GnRH-positive neurons during early development (Spilker et al. 2016). This is consistent with the allelic variants reported in humans being heterozygous, associated with other verified causative gene mutations and not fully segregating with the clinical phenotype (Pitteloud et al. 2007a; Xu et al. 2011). Thus, the extreme rarity of this gene variants together with these reported observations question the role of *NSMF* in the pathogenesis of CHH and, at the very least, make monogenic causation of CHH by loss-of-function mutations very unlikely.

### ***WDR11***

Synergistic with *EMX1* (a transcription factor encoded by Empty Spiracles Homeobox 1—*EMX1*) in the development of murine olfactory neurons, WD Repeat Domain 11 shuttles between nucleus and cytoplasm to activate transcription (Kim et al. 2010). Initially expressed widely during central nervous system (CNS) development—but particularly in the regions involved in the development of hypothalamic GnRH neurons (Kim et al. 2010)—it later localizes just to olfactory structures, cerebellum and hippocampus. In 2010, Kim et al. described several missense heterozygous variants in *WDR11* domains important for protein–protein interaction, in patients with both KS and nCHH (Kim et al. 2010), alone or associated with mutations in other known genes (Kim and Layman 2011), suggesting the possibility of oligogenic or AD inheritance. A *WDR11* variant has also been reported in association with a *PROKR2* variant in patient with CPHD and absent pituitary stalk (McCormack et al. 2017).

### ***FEZF1***

*FEZF1* is a zinc finger protein acting as a repressor of transcription during the embryogenesis of olfactory structures; enabling olfactory and GnRH neurons to access the brain. Loss-of-function missense (affecting the central C<sub>2</sub>H<sub>2</sub> motif

necessary for protein stability) or nonsense autosomal recessive (AR) allelic variants of this gene are associated with severe and highly penetrant KS phenotypes (Kotan et al. 2014). Furthermore, KO mice replicate the human phenotype, with hypoplastic olfactory structures and CHH (Hirata et al. 2006; Watanabe et al. 2009). Nevertheless, the lack of new reported human variants since then suggests either the extreme rarity of *FEZF1* variants in CHH/KS pathogenesis or that other mutational events might explain the reported phenotypes in the two independent consanguineous index families.

### ***CCDC141***

*CCDC141* is a cytoskeletal scaffolding protein, with a role in cellular motility. It is expressed in GnRH neurons and its missense allelic variants have been reported in association with nCHH, incomplete penetrance and possible reversal (Turan et al. 2017), complex inheritance patterns observed in families described seeming to support oligogenicity. In an animal model, *CCDC141* was shown to be important for GnRH neuron migration along olfactory axon fascicles, but without affecting development of the fascicles themselves, consistent with the postulated human phenotype (Hutchins et al. 2016).

### **Netrin signalling (*DCC* and *NTN1*)**

Netrin-1 (*NTN1*) is an axon guidance secreted protein, containing an *Anosmin1*-like fibronectin type-III (FN3) domain. *DCC* encodes its receptor, with the animal model exhibiting a KS-like phenotype (Schwartz et al. 2004; Lakhina et al. 2012). In a study of 133 CHH patients, six patients harboured heterozygous *DCC* missense variants and two harboured heterozygous *NTN1* missense variants. Five of six subjects had olfactory impairment and all had severe GnRH deficiency (Bouilly et al. 2018). Although inheritance was compatible with AD transmission, some patients also harboured mutations in other CHH genes and two of them had variants in both *DCC* and *NTN1*, thus also supporting oligogenic inheritance. In vitro studies of these variants revealed altered intracellular signalling associated with alterations in cell morphology due to defective binding (Bouilly et al. 2018). Interestingly, alterations in *DCC* and *NTN1* signalling had already been associated with congenital mirror movements and corpus callosum agenesis (Srour et al. 2010; Marsh et al. 2017),

### ***SOX10* and *TUBB3***

*SOX10* is a transcription factor involved in the early development of neural crest cells, which are a population of multipotent precursor cells arising from the neural tube that

differentiate into various specific cell types. *SOX10* also has critical influence on auditory function through its expression in the melanocytic intermediate cells of the cochlear stria vascularis during early development of the inner ear (Breuskin et al. 2009). Mutations are classically associated with Waardenburg syndrome, a rare disorder characterized by sensorineural congenital hearing loss and abnormal pigmentation of the hair, skin and eye (Pingault et al. 2010). However, *SOX10* loss-of-function mutations have also been found in nearly 40% of KS patients having with hearing impairment (Pingault et al. 2013).

Given that the prevalence of deafness in KS individuals is estimated to be only 5% and that *SOX10* mutations are rare in KS individuals with normal hearing, this relationship appears highly significant. *SOX10* mutations were shown to affect olfactory ensheathing cells during early embryonic development of the peripheral olfactory system in mouse models, leading to the disrupted development of the olfactory neurons as well as impaired migration of GnRH neurons, thus underpinning the KS phenotype (Pingault et al. 2013).

Similar to *SOX10*, genetic alterations in *TUBB3* have been associated with disrupted migration of neural crest cells. The *TUBB3* protein is a member of tubulin family and its alterations are responsible for a multiple neurological complex syndrome (Whitman et al. 2016; Ceylan et al. 2017; Huang et al. 2018). A single missense mutation (p.E410K) of *TUBB3* was also associated with KS and severe peripheral neuropathy, having with an AD inheritance pattern with strong genotype–phenotype correlation (Chew et al. 2013; Balasubramanian et al. 2015; Nakamura et al. 2018).

#### **AMH and AMHR2**

AMH belongs to the TGF- $\beta$  family of proteins and its cognate receptor is AMHR2. Signalling in this pathway plays a pivotal role in sex differentiation, with homozygous loss-of-function mutations leading to persistence of Mullerian ducts in males. However, later in gestation, it also plays a role in the regulation of GnRH neuron migration towards the brain, through an autocrine mechanism intervening in axonal growth and pathfinding (Liu et al. 2004). In fact *AMH* is expressed in migratory GnRH neurons in both mouse and human fetuses, and *Amhr2*-deficient mice show abnormal development of the peripheral olfactory system and defective GnRH cells migration, with reduced fertility in adults (Cimino et al. 2016; Malone et al. 2019).

Only recently, heterozygous missense *AMH* allelic variants in the N-terminal pro-protein domain have been found in both KS and nCHH subjects and an in-frame 27-nucleotide deletion in the catalytic intracellular serine/threonine domain of the receptor *AMHR2* found in a patient with nCHH. These variants are present in both male and female

subjects with absent or partial puberty, one of whom also exhibited high-arched palate (Malone et al. 2019). These findings are consistent with AD inheritance with variable expressivity (variability in spontaneous puberty and even fertility since the same variants were found in parents), although oligogenicity obviously cannot be excluded. Therefore, only a wider genetic study on a large patient cohort will clarify the role of these genes in CHH/KS pathogenesis.

#### **NDNF**

*NDNF* (neuron-derived neurotrophic factor) is involved in neuron survival, migration and neurite outgrowth (Kuang et al. 2010). It belongs to the FN3 superfamily of genes, which encode proteins typically involved in protein–protein interactions related to cell adhesion, migration and embryonic development (Bencharit et al. 2007). *Ndnf* deficient mice showed anomalies in GnRH neuron migration to the hypothalamus and development of the olfactory axonal scaffold, findings that were further confirmed using a Zebrafish *ndnf* morpholino-based knockdown model (Messina et al. 2020).

This is the most recent gene to be associated with CHH, with four different heterozygous variants reported in different subjects having KS with severe GnRH deficiency. Pedigree analysis of the affected kindreds is consistent with AD inheritance with variable expressivity and incomplete penetrance, although oligogenicity cannot be excluded. Only one of the allelic variants was missense, two were nonsense mutations and one was a frameshift that introduced a premature stop codon. Only the latter three variants—associated with a truncated protein—resulted in a loss of function in vitro (Messina et al. 2020).

### **GnRH neuron and gonadotroph differentiation and fate specification**

#### **FGFR1 synexpression group**

*FGFR1* is a member of the tyrosine kinase receptor superfamily of proteins and gain-of-function mutations are associated with craniosynostosis. *Fgfr1* plays a role in GnRH neuron proliferation and migration to the hypothalamus as well as directly promoting olfactory bulb development. Accordingly, loss-of-function *FGFR1* mutations can lead to defective GnRH neuron migration through abnormal olfactory bulb morphogenesis, although some individuals express a “pure” neuroendocrine nCHH phenotype without non-reproductive anomalies, implying a putative role in the control of GnRH secretion that remains to be defined.

Indeed, *FGFR1* was the first gene in which inactivating mutations were found in both normosmic CHH and KS. Loss-of-function mutations in *FGFR1* have been identified

in approximately 10% of CHH patients, with the majority of pathogenic variants being missense mutations (Dodé et al. 2003). It has a potentially AD inheritance, albeit associated in many kindreds with markedly incomplete penetrance, interfamilial variability (Pitteloud et al. 2006) and oligogenicity (Pitteloud et al. 2007a). Phenotypically, affected individuals are enriched with skeletal manifestations, including craniofacial clefting, digit anomalies (syndactyly, oligodactyly and clinodactyly) and dental agenesis (Jarzabek et al. 2012; Costa-Barbosa et al. 2013).

Loss-of-function *FGFR1* mutations were recently also implicated in Hartsfield syndrome, characterized by holoprosencephaly and split hand/foot malformation (absent or partial development of the central rays of the hands or feet) (Simonis et al. 2013), consistent with the observation that, among the rare subgroup of CHH patients with split hand/foot malformation, around 90% harbour *FGFR* mutations (Villanueva et al. 2015).

FGF8 functions as a potent ligand for FGFR1. *FGF8* is expressed in the diencephalon and prospective hypothalamus during embryonic development (McCabe et al. 2011a). It is a critical morphogen for GnRH neuron fate specification and olfactory system development. Targeted transgenic mice lacking olfactory placode *Fgf8* expression fail to develop GnRH neurons. In humans, *FGF8* mutations account for no more than 1% of CHH cases, with an apparently AD mode of inheritance. Cleft lip or palate and other midline defects have been reported in subjects harbouring the mutation. *FGF8* mutations have also been found to be associated with recessive holoprosencephaly, craniofacial defects and hypothalamic–pituitary dysfunction (McCabe et al. 2011a). Studies in mice revealed a decrease in 30–50% of total GnRH neurons in those harbouring monogenic heterozygous *Fgf8* mutations, whereas mice with digenic *Fgfr1/Fgf8* mutations suffered a greater reduction in GnRH neurons, suggesting a high degree of interaction between these FGF signalling factors in promoting tropic support for the emergence of GnRH neurons in the olfactory placode (Chung et al. 2010).

*Fgf17* is co-expressed with *Fgf8* in the olfactory placode and is thus considered a member of the *Fgf8* synexpression group (Miraoui et al. 2013). Possessing a high degree of homology to *FGF8*, *FGF17* shares similar signalling effect through isoform FGFR1c and is thus implicated in GnRH neuron embryonic morphogenesis (Olsen et al. 2006). Heterozygous *FGF17* mutations have been identified in three patients with CHH (2 KS and 1 normosmic) (Miraoui et al. 2013).

IL17RD is a single transmembrane glycoprotein with sequence resembling the intracellular domain of the interleukin-17 receptor. It is an antagonist of the FGF signalling pathway, both at the level of the FGF receptors, including FGFR1, as well as the downstream components of the Ras–ERK1/2 pathway. Having close spatiotemporal

interdependence with *FGF8* in the olfactory placode suggests an equally critical role for the initial phase of GnRH neuron fate specification (Miraoui et al. 2013). Additionally, it likely exerts modulatory effect on embryonic optic development via the FGF8–FGFR1c pathway, consistent with the association of *IL17RD* mutations in KS with congenital hearing impairment. Phenotypic expression is likely only in the presence of biallelic *IL17RD* mutations or of oligogenicity, rather than from a single allelic defect (Miraoui et al. 2013).

*SPRY4* encodes sprout homolog 4, a protein that exerts antagonistic effect on FGF signalling via inhibition of the receptor-transduced mitogen-activated protein kinase (MARK) signalling pathway. It is located upstream of RAS gene activation and disrupts the formation of active GTP-RAS (Hanafusa et al. 2002). In murine studies, *SPRY4* is shown to regulate neurite outgrowth in PC12 cells, a widely utilized model of neuronal differentiation, and hippocampal neurons. Knockout mice exhibit craniofacial and limb defects (Taniguchi et al. 2007) (Hausott et al. 2012). Close to 4% of a CHH cohort in one study were found to harbour *SPRY4* mutations, with two-thirds manifesting impaired olfaction.

*DUSP6* encodes a member of the dual specificity protein phosphatase which inactivates members of the mitogen-activated protein (MAP) kinase superfamily. In mice studies, *Dusp6* mutant alleles result in skeletal dwarfism, craniosynostosis and hearing loss (Li et al. 2007). Human *DUSP6* mutations account for no more than 1% of CHH subjects and are found in both KS and normosmic CHH phenotypes, with other associated non-reproductive features being hearing impairment and abnormal dentition (Miraoui et al. 2013).

*FLRT3* encodes a member of the fibronectin leucine-rich transmembrane (FLRT) family that interacts with FGFR during embryogenesis. The transmembrane cell adhesion protein is characterized by a cluster of leucine-rich repeats and one fibronectin type-III domain within the extracellular matrix. As an axon guidance-related factor and enhancer of the FGF network, it promotes the activation of FGF signalling via ERK phosphorylation and neurite outgrowth in rat neuronal cells (Böttcher et al. 2004; Robinson et al. 2004). *FLRT3* variants were found in three unrelated KS individuals (two females and one male) manifesting CHH with partial or complete absence of puberty (Miraoui et al. 2013).

*KLB* encodes  $\beta$ -Klotho, a type I single-pass transmembrane protein that functions as the primary high-affinity receptor for FGF21, which in turn exerts its effect via signalling through the  $\beta$ -klotho/FGFR1c receptor complex (Ming et al. 2012). In mice, lack of *Klb* results in defective FGF21-mediated GnRH secretion by hypothalamic GnRH neurons, leading to pubertal delay, altered oestrous cyclicity and subfertility (Xu et al. 2017). In a cohort of CHH subjects, 4%

were found to harbour heterozygous loss-of-function *KLB* mutations; of the seven heterozygous variants identified in the probands, six were missense variants and one was an in-frame deletion (Xu et al. 2017). As the FGF21/KLB/FGFR1 pathway also mediates metabolic processes, it may reinforce a linkage between metabolic and reproductive processes.

### **CHD7**

*CHD7* encodes chromodomain helicase DNA-binding protein 7, which is expressed in a variety of foetal tissues including the developing brain. It is one of two CHH genetic loci shared with CHARGE syndrome, a rare developmental disorder of autosomal dominant inheritances, characterized by iris coloboma, congenital heart disease, choanal atresia, mental and growth retardation, genital hypoplasia, and ear malformations or deafness (Jongmans et al. 2006).

CHH occurs in around 2/3 of patients carrying *CHD7* mutations (Jongmans et al. 2006); conversely, only 6% of patients CHH (with or without anosmia) harbour *CHD7* mutations (Kim et al. 2008a). This is not surprising given the overlapping features of olfactory impairment and CHH observed in these two groups of patients (Jongmans et al. 2009). Therefore, while multisystem involvement and large de novo deletions characterize classical CHARGE syndrome, *CHD7*-associated CHH appears to result from inherited point mutations (Kim et al. 2008a; Balasubramanian et al. 2014).

### **IGSF10**

*IGSF10* is a member of the immunoglobulin superfamily, which appears to form part of a complex system of chemostatic agents directing the early migration of GnRH neurons from the nasal region to the hypothalamus (Wray 2010); aberrant signalling due to pathogenic *IGSF10* variants can lead to impaired GnRH neuron migration to the hypothalamus (reduced population or mis-timed arrival), as supported by an *Igsf10* knockdown-zebrafish model, wherein disruption of immature GnRH neuron migration was demonstrated. The authors postulated that variable phenotypes may manifest according to the burden of other associated mutations (oligogenicity), ranging from self-limited delayed puberty to CHH (Howard et al. 2016).

### **SOX2**

*SOX2*, *SRY (Sex Determining Region Y)-box 2* gene, encodes a transcriptional regulator in pluripotent stem cells, required for maintaining their pluripotency, as well as directing their neural differentiation (Zhang and Cui 2014). *Sox2* is important in the development of the anterior pituitary gland in the mouse. Indeed, although *Sox2*<sup>-/-</sup> null embryos die shortly

after implantation (Avilion et al. 2003), heterozygous mutant mice showed a variable altered size and shape of the pituitary gland and a significant reduction in pituitary hormone content in affected mice (Kelberman et al. 2006).

Human *SOX2* mutations are associated with bilateral anophthalmia or severe microphthalmia, with anterior pituitary hypoplasia, CHH and genital abnormalities in males (Fantès et al. 2003; Williamson et al. 2006; Kelberman et al. 2006; Jayakody et al. 2012). Additional forebrain defects include hypoplasia of the corpus callosum, hypothalamic hamartoma and hippocampal malformation (8, 10), frequently associated with additional abnormalities, including oesophageal atresia, sensorineural hearing loss and learning difficulties (Sisodiya et al. 2006; Kelberman et al. 2006).

### ***NROB1* (encoding *DAX-1*)**

Encoded by *NROB1*, *DAX-1* (dosage sensitive sex reversal adrenal hypoplasia, critical region on the X-chromosome, gene 1) is an orphan nuclear receptor expressed in the adrenals, gonads, pituitary gonadotrophs and ventromedial hypothalamus, and therefore plays a key role in adrenal and reproductive development. Accordingly, *NROB1* mutations result in an X-linked form of primary adrenal hypoplasia congenital, associated with CHH and impaired spermatogenesis (Muscatelli et al. 1994). Most patients present first with neonatal adrenal insufficiency and, subsequently, in adolescence with pubertal delay due to CHH arising from a combined hypothalamic, pituitary and gonadal defect (Habiby et al. 1996). However, rare cases of late onset in adulthood have been reported (Kyriakakis et al. 2017).

## **GnRH neuron activation and networking**

### ***Kisspeptin* and *KISS1R***

*Kisspeptin* (*KISS1*) and its receptor *KISS1R* (formerly *GPR54*) were identified as causal genes for CHH in 2003 (de Roux et al. 2003; Seminara et al. 2003) and 2012 (Topaloglu et al. 2012), respectively. The identification of the hypothalamic kisspeptin neuronal network has transformed our perception of the control and activation of GnRH secreting neurons at puberty. Indeed, kisspeptin (formerly known as metastin) is a strong activator of the hypothalamic–pituitary–gonadal axis in both human and animal models. *KISS1* encodes four different products, whose binding to *KISS1R* is independent of their length (Vezzoli et al. 2016).

*KISS1* is expressed in periventricular and arcuate nuclei (Gottsch et al. 2004) and is the main regulator of GnRH neuron activation and hormone secretion, as demonstrated by the precocious puberty phenotype associated with a gain-of-function mutation in *KISS1R* (Teles et al. 2008). Both *Kiss1* and *Kiss1r* knockout mice phenocopy human CHH

(Seminara et al. 2003; Funes et al. 2003; de Tassigny et al. 2007). Since these two genes play no role in GnRH neuron migration and development they produce a “pure neuroendocrine” nCHH phenotype with an AR mode of inheritance (de Roux et al. 2003; Seminara et al. 2003; Cerrato et al. 2006; Topaloglu et al. 2012). So far, there are reports of both nonsense mutations impairing receptor function (de Roux et al. 2003) and membrane targeting (Seminara et al. 2003), and missense mutations affecting domains essential for the activation of KISS1R (Seminara et al. 2003), its expression on the membrane, or its signalling (Semple et al. 2005; Tenenbaum-Rakover et al. 2007). Both a deletion and a missense variant found in the third intracellular loop decreased KISS1R signalling (de Roux et al. 2003; Semple et al. 2005).

### TAC3 and TACR3

*TAC3* encodes a tachykinin called Neurokinin B (NKB), and *TACR3* its cognate receptor (a GPCR of the rhodopsin family). Both are expressed mainly in the arcuate nucleus of the hypothalamus (together with *KISS1*) in KNDy (kisspeptin–neurokinin–dynorphin) cells projecting to GnRH neurons (Goodman et al. 2007; Navarro et al. 2009; Wakabayashi et al. 2010), and are believed to play an important role in GnRH activation during puberty, foetal life and perinatal minipuberty (Gianetti et al. 2010). They were first associated with AR transmission of CHH in 2009, with in vitro validated allelic variants being missense (Topaloglu et al. 2009; Gianetti et al. 2010), nonsense (Gianetti et al. 2010), or splice-site variants (Young et al. 2010), impairing receptor signalling or post-translational modifications necessary for correct activation of the tachykinin motif.

Rat models initially gave contrasting results on the stimulatory effect of NKB, probably due to the necessity for physiological levels of sex steroids for its stimulatory effect to be expressed, which was subsequently confirmed in many species (Navarro 2013). Human mutations are associated with nCHH, often with cryptorchidism and micropenis, but potentially with greater propensity for reversal (Gianetti et al. 2010).

### Direct secretion and action of GnRH

#### *GNRHR* and *GNRH1*

Gonadotropin-releasing hormone (encoded by *GNRH1*) and its receptor (encoded by *GNRHR*) are the primary actors in the regulation of human reproduction. The pulsatile secretion of GnRH in the median eminence and the interaction with its receptor on pituitary gonadotrophs modulate the release of gonadotropins (Seeburg and Adelman 1984). Thus far, *GNRH1* is the only hypothalamic releasing hormone gene found mutated in humans. Although GnRH1 was

the very first candidate gene for CHH (Weiss et al. 1991), mutations were only identified in humans 10 years ago due to their exceptional rarity (Bouligand et al. 2009; Chan et al. 2009). By contrast, *GNRHR* was among the first three genes to be associated with CHH (de Roux et al. 1997; Layman et al. 1998).

GnRH resistance mediated by deleterious variants of *GNRHR* has a heterogeneous phenotype and inconstant expressivity causing both CHH and AHH, with mixed AR and oligogenic inheritance (Cerrato et al. 2006), and with most AR cases exhibiting compound heterozygosity. It is a common cause of familial nCHH with more than 25 variants reported in the literature (Cioppi et al. 2019) and can also produce incomplete phenotypes with milder or partial disease (de Roux et al. 1999; Pitteloud et al. 2001; Cangiano et al. 2019; Cioppi et al. 2019), including possible reversal forms (Raivio et al. 2007). The majority of the variants found in humans are missense variants that variably impair GnRH signalling through abnormal intracellular trafficking, hormone binding, G-protein coupling or receptor expression (Bianco and Kaiser 2009).

*Gnrh1* knockout mice show a similar phenotype to human CHH (Pask et al. 2005; Wu et al. 2010). In contrast, human *GNRH1* gene allelic variants (nonsense variants with AR or oligogenic inheritance), are exceptionally rare and are all located in the secreted decapeptide itself (Bouligand et al. 2009; Chan et al. 2009; Megen et al. 2016). In the mouse model, mutations of *Gnrh1* produce infertility, sexual immaturity, and tooth and mineralization defects (Cattanach et al. 1977; Tiong et al. 2007). However, in humans, *GNRHR* and *GNRH1* mutations are associated with nCHH without non-reproductive defects, since they have no known role in normal embryonic development (de Roux et al. 1997; Beranova et al. 2001; de Roux 2006).

### Actual or functional energy-deficit CHH

#### *LEP* and *LEPR*

Leptin, encoded by *LEP* (formerly *Ob*), is a hormone secreted by white adipose tissue, in proportion to the total fat mass and inversely regulated by exercise. It acts through the leptin receptor, encoded by *LEPR* (formerly *Db*) and belonging to the class 1 cytokine receptor family. Rodents harbouring homozygous mutations in *Lep* and *LepR* exhibit early-onset morbid obesity, hyperphagia and decreased energy expenditure (Zhang et al. 1994; Lee et al. 1996). Parabiosis experiments performed in the pre-genetic era, wherein the blood circulations of *Lep* and *LepR* mice were linked, famously resulted in dramatic transformation of the *Lep* mouse from a hyperphagic obese phenotype into one of starvation with cessation of food intake, the *LepR* phenotype remaining necessarily unchanged.

In humans, leptin signalling deficiency caused by loss-of-function *LEP* or *LEPR* mutations is an extremely rare cause of monogenic obesity and CHH. Notably, individuals with heterozygous *LEP* or *LEPR* mutations do not exhibit pubertal or reproductive disorders, as only one functional copy is required for normal reproductive function (Clément et al. 1998). In a hyper-selected cohort of 300 subjects with hyperphagia and childhood-onset obesity, the prevalence of pathogenic *LEPR* mutations was only 3% (Farooqi et al. 2007b), whereas the prevalence of homozygous leptin variants in general population is estimated to be 1 in 4.4 Mio. (Nunziata et al. 2017).

Affected individuals suffer from severe obesity as a consequence of relentless hyperphagia. Furthermore, as a clear indication of the critical link that leptin serves between nutritional status and hypothalamic–pituitary–gonadal function, they also manifest CHH and fail to undergo normal puberty. In leptin-deficient subjects, recombinant leptin therapy was effective in reversing HH and restoring gonadal and sexual function (Farooqi et al. 1999; Farooqi 2002). Moreover, recombinant leptin also restored menstrual cyclicality and reproductive function in women with hypothalamic amenorrhea (HA) and low fat mass due to energy deficit (Welt et al. 2004).

Although the effect of leptin on the gonadotropic axis is remarkable, GnRH neurons do not themselves express *LEPR*, their activity being instead modulated indirectly via forebrain neurons expressing *LEPR* that are afferent to GnRH (Quennell et al. 2009). Although *LEPR* is also expressed in a subset of kisspeptin neurons (Smith et al. 2006), the main site of leptin action to regulate reproduction is not directly on kisspeptin neurons, but rather through cells in the ventral premammillary nucleus (PMV) (Cravo et al. 2013; Cavalcante et al. 2014; Iacovazzo et al. 2016). Finally, it was recently demonstrated that PACAP (pituitary adenylate cyclase-activating polypeptide) secreted by leptin-responsive neurons in the hypothalamic ventral premammillary nucleus (PMV), also serves as a mediator for leptin to exert its reproductive role (Ross et al. 2018).

HA is a reasonably common condition exhibited by women with energy deficit due to overexercising or restriction of food intake, short of frank anorexia nervosa that requires nutritional rehabilitation. The observation that only a proportion of women facing similar degrees of energy deficit develop HA was recently explained by the observation that women with HA are enriched with deleterious heterozygous variants of CHH genes compared with normally menstruating controls (Caronia et al. 2011).

By contrast, energy-deficit functional CHH seems to be much rarer in males (who are paradoxically more predisposed to develop functional CHH in relation to obesity). Moreover, no convincing enrichment with deleterious CHH

alleles has been demonstrated in these men (Dwyer et al. 2019a).

## Defects of gonadotropin secretion or action

### *PCSK1*

*PCSK1* encodes neuroendocrine convertase, whose deficiency results in impaired prohormone processing; in particular this enzyme is responsible for processing pro-opiomelanocortin in the corticotroph to produce adrenocorticotrophic hormone and lipotropin. Rare cases of compound heterozygosity and homozygosity for mutation in the *PCSK1* gene have been reported in subjects with associated features of early-onset severe obesity and hyperphagia with CHH (Jackson et al. 1997; Farooqi et al. 2007a).

### *LHB* and *FSHB*

Biallelic inactivating mutations in the specific  $\beta$ -subunits of LH and FSH have been described as rare causes of men and women presenting with reproductive disorders and are characterized by an unusual hormonal profile, not typical of CHH, wherein levels of only one of the two gonadotropins are low, whilst levels of the other are normal or even elevated. Homozygosity and compound heterozygosity with AR mode of transmission have been reported (Lofrano-Porto et al. 2007; Basciani et al. 2012).

Men with pathogenic *LHB* variants express normal sexual differentiation, implying in utero responsiveness to chorionic gonadotropin (hCG), but exhibit pubertal failure, Leydig cell hypoplasia and spermatogenic arrest from the lack of LH-stimulated testosterone secretion (Basciani et al. 2012). Accordingly, restoration of testicular growth and spermatogenesis with hCG therapy was described in a man with homozygous missense *LHB* mutation (Valdes-Socin et al. 2009). By contrast, affected women may undergo normal puberty, but then manifest secondary amenorrhoea and anovulatory infertility (Lofrano-Porto et al. 2007).

In human and mouse studies, FSH is essential for qualitatively and quantitatively normal sperm production in males as well as for normal female pubertal development and fertility via the regulation of ovarian follicular development. Deleterious *FSHB* mutations are autosomal recessive, with men harbouring biallelic *FSHB* mutations undergoing normal puberty, but remaining azoospermic, whereas women present with abnormal pubertal development and primary amenorrhoea (Layman et al. 1997, 2002).

## Gonadotroph differentiation and CPHD

An entire subset of genes act through impairment in the development, differentiation, or fate specification of pituitary

cells, typically generating a complex phenotype with CPHD. However, many genes previously associated with CHH (such as *PROKR2*, *FGF8*, *FGFR1*, *SOX2*, *WDR11*) have recently also been associated with CPHD (including septo-optic dysplasia—SOD) and vice versa (McCabe et al. 2011b, 2013, 2015; Raivio et al. 2012; Jayakody et al. 2012; Izumi et al. 2014), sometimes even interacting in an oligogenic manner (McCormack et al. 2017).

#### **LHX4**

*LHX4*, encoding Lim Homeobox gene 4, is highly conserved in evolution and plays an important role as a transcriptional regulator of embryonic development. Human *LHX4* encodes a 390-amino acid protein that has a tandem pair of LIM domains and one homeodomain, and is highly homologous to the human LHX3 protein, except for the N-terminal region (Kawamata et al. 2002). As demonstrated in null mice models, both *Lhx3* and *Lhx4* regulate proliferation, differentiation and fate specification of pituitary-specific cell lineages, dictating pituitary gland identity and controlling developmental decisions at multiple steps of organogenesis (Sheng et al. 1997).

In 2001 Machinis et al. described *LHX4* heterozygous mutations in four affected members of a French family with a syndrome of autosomal dominant short stature, pituitary and cerebellar defects and abnormalities of the sella turcica (Machinis et al. 2001). Following this first report, other authors reported allelic variants in *LHX4* gene in different domains of the protein—all associated with hypopituitarism, including CHH. Nevertheless, *LHX4* mutations remain a relatively rare cause of CPHD (Pfaeffle et al. 2008).

#### **HESX1**

*HESX1* is another homeobox gene and an important transcription repressor involved in the morphogenesis of pituitary gland, forebrain, hypothalamus and optic nerve (Dattani et al. 1998). It has also been shown to be a transcriptional target of *OTX2*. Variable inheritance patterns and penetrance are described, leading to a range of phenotypic manifestations including KS, isolated GHD and CPHD, with or without associated forebrain and ocular defects (McNay et al. 2007; Newbern et al. 2013; Fang et al. 2016a). *HESX1* mutations have been identified in approximately 1% of CHH and septo-optic dysplasia patients. Both *HESX1* recessive and dominant variants may underlie CPHD (Romero et al. 2011).

#### **PITX2 and GATA2**

*PITX2* is another homeobox gene. The PITX2 protein plays a critical role in pituitary organogenesis, including the growth of Rathke's pouch and maintaining expression of

the transcription factors PROP1 and HESX1, and later in the fate specification and expansion of gonadotrophs and PUO1F1 (PIT1) lineages within the ventral and caudomedial anterior pituitary (Dattani and Robinson 2000; Suh et al. 2002). AD inheritance of *PITX2* produces Axenfeld–Rieger syndrome, with alterations of the anterior segment of the eyes, dental abnormalities and hypopituitarism (Seifi and Walter 2018), including potentially CHH. In mouse models, diminished expression of gonadotrope markers *Lhb*, *Fshb* and *Gnrhr* in *Pitx2* hypomorphs point to the importance of *Pitx2* in cell fate specification (Suh et al. 2002). Moreover, *Pitx2* is necessary to drive the cascade of transcription factors (starting with *Gata2*) in cell lineage fate specification and proliferation (Suh et al. 2002).

*GATA2* is expressed in the developing pituitary as well as adult gonadotropes and thyrotropes. *Gata2* is genetically downstream of transcription factor *Pitx2* (Suh et al. 2002). In pituitary-specific knockout mice, gonadotropin secretion was reduced both basally and in response to castration challenge, albeit fertility was preserved (Charles et al. 2006). Consistent with this finding, *GATA2* was demonstrated to participate in the modulation of *LHβ* gene promoter and *Gnrhr* promoter expression by other investigators (Lo et al. 2011; Schang et al. 2013).

#### **PROP1**

*PROP1* is the most frequently mutated gene implicated in non-syndromic CPHD, accounting for up to 50% of familial cases. It acts both as a repressor in down-regulation of *HESX1* and as an activator of *POU1F1*, possibly via Wnt/β-catenin signalling, in the differentiation of anterior pituitary cells including gonadotropes (Dasen and Rosenfeld 2001; Olson et al. 2006). AR mode of inheritance of loss-of-function *PROP1* variants is observed in kindreds. Compared to growth hormone and thyrotropin deficiencies, which typically manifest early in life, gonadotropin deficiency may only evolve to present later as pubertal failure during adolescence (Turton et al. 2005; Kelberman et al. 2009).

#### **LHX3**

*LHX3* belongs to the Lin11, Isl-1 and Mec-3 (LIM) homeodomain protein family of transcription factors. It is expressed in the neural tube and Rathke's pouch and has an essential role in regulating pituitary development and the differentiation of gonadotropes, thyrotropes, somatotropes and lactotrophs, as well as in the organisation of spinal cord neurons (Tsuchida et al. 1994; Bach et al. 1995). Mutations of *LHX3* are AR, with majority found to be missense or nonsense mutations. Besides anterior pituitary hormone deficiencies, affected individuals may present with short neck with limited neck rotation and vertebral abnormalities (Bhangoo

et al. 2006; Pfaeffle et al. 2007). As *LHX3* variants disrupt development of inner ear, affected individuals may also manifest a varying degree of hearing loss (Bonfig et al. 2011; Pozza et al. 2012).

### SOX3

*SOX3* belongs to the SRY-related HMG transcription factor family. It is expressed widely in the central nervous system with a critical role in hypothalamic–pituitary morphogenesis. Duplications and deletions in the polyalanine tract of *SOX3* have been shown to result in varying severity of anterior pituitary deficiencies (Alatzoglou et al. 2011). In a recent Japanese genome-wide copy number analysis and systematic mutation screening study, polyalanine deletions in *SOX3* were described for the first time as a genetic cause of normosmic CHH among a cohort of patients (Izumi et al. 2014).

### OTX2

Orthodenticle Drosophila homolog 2 (*OTX2*) is a homeobox family transcription factor. *Otx2* is expressed in anterior neuroectoderm during early embryogenesis development and participates critically in the formation of the rostrum, midbrain and eyes (Kurokawa et al. 2004; Henderson et al. 2009). Around 1/3 of subjects harbouring *OTX2* mutations were found to have pituitary insufficiency, including CHH (Schilter et al. 2011). *OTX2* mutations also account for 2–3% of cases of congenital anophthalmia/microphthalmia (Wyatt et al. 2008). The variable penetrance of *OTX2* variants, even within the same family (Gorbenko Del Blanco et al. 2012), is likely determined by oligogenicity and environmental factors (Tajima et al. 2013). In mice, heterozygous *Otx* male embryos had a substantial reduction in the total GnRH neuron population due to impaired neuronal migration from the nasal placode, resulting in subfertility and compromised production of luteinising hormone in adulthood; by contrast, heterozygous female mutants did not survive to adulthood (Diaczok et al. 2011; Hoffmann et al. 2019).

### GLI2

*GLI2* is a zinc finger transcription factor involved in the regulation of the Sonic Hedgehog signal transduction, expressed in the ventral diencephalon and oral ectoderm, which induces *BMP4* and *FGF8* expression and pituitary progenitors, respectively. Mutant mice with inactivating *Gli2* manifest hypoplasia of the anterior pituitary gland and anomalies of the midline central diencephalon. In humans, *GLI2* inactivating mutations are associated with a phenotype at the milder end of the holoprosencephaly spectrum, including pituitary gland malformation and craniofacial defects as

well as varying degree of hypopituitarism (isolated GHD through to CPHD) (Kevelam et al. 2012). The inheritance pattern follows that of autosomal dominant with incomplete penetrance and variable expressivity (Kevelam et al. 2012).

## Syndromes of CHH with cerebellar ataxia

A group of genes discovered with next-generation techniques have been associated with neurological syndromes (especially ataxia) that are usually associated with CHH.

*POLR3A* and *POLR3B* encode catalytic subunits of RNA polymerase and AR inheritance of mutations results in abnormal central nervous system white matter: the hypomyelinating leukodystrophy (Daoud et al. 2013) with prominent cerebellar dysfunction, oligodontia and CHH (Bernard et al. 2011; Tétreault et al. 2011).

*OTUD4*, *RNF216* and *STUB1* encode proteins involved in protein quality control. AR or oligoallelic inheritance of variants in these genes leads to disordered ubiquitination, determining the Gordon Holmes syndrome (Seminara et al. 2002; Margolin et al. 2013; Shi et al. 2014), which is characterized by hypogonadotropic hypogonadism (both of hypothalamic and pituitary origin), spinocerebellar ataxia, spasticity and dementia. AR allelic variants in *PNPLA6* can also produce either Gordon Holmes syndrome, or Boucher–Neuhäuser syndrome (lacking spasticity, but instead exhibiting visual impairment due to chorioretinal dystrophy). *PNPLA6* encodes an enzyme necessary for synthesis of acetylcholine and mutations are usually located in the C-terminal phospholipid esterase domain, thus inactivating its catalytic function (Synofzik et al. 2014; Topaloglu et al. 2014).

## Other syndromic forms

### DMXL2

Encoding a synaptic scaffold protein for the regulators of the GTPase Rab3a expressed in exocytosis vesicles of GnRH neurons and gonadotrophs, *DMXL2* is associated with a polyendocrine-polyneuropathy syndrome, with ataxia and dysarthria, characterized by mild CHH, central hypothyroidism, peripheral demyelinating neuropathy and severe hypoglycaemia that later degenerated into insulin-dependent diabetes mellitus (Tata et al. 2014). It has AD transmission and a mild reproductive phenotype with incomplete puberty and normal sense of smell. In heterozygous knockout mice it is associated with a reduction in the number of GnRH neurons, retardation of puberty and impaired fertility (Tata et al. 2014). Functional studies found *DMXL2* to be involved in both constitutive and glucose-induced secretion of insulin by pancreatic beta cells.



### **TBX3**

*TBX3* is a member of the family of transcriptional factors sharing a DNA-binding domain known as T-box. As it is expressed in pituitary gland, gonads and genital tubercles, as well as mammary glands and limbs, *TBX3* haploinsufficiency results in ulnar–mammary syndrome (UMS), a rare AD characterized by under-developed external genitalia, delayed puberty, ulnar ray defects and hypoplasia of nipples and apocrine glands (Bamshad et al. 1997). Heterozygous variants of *TBX3* have been described in two unrelated families with nCHH and pituitary hypoplasia, which is in line with the high prevalence of pubertal delay reported in the UMS literature (Sasaki et al. 2002; Galazzi et al. 2018).

### **SMCHD1**

SMCHD1 is an epigenetic repressor, intervening in the inactivation of the X-chromosome (Blewitt et al. 2008) and is especially expressed in immature olfactory neurons (Nickell et al. 2012). In 2017 Shaw et al. identified heterozygous missense *SMCHD1* mutations in the GHKL-type ATPase domain in 84% of patients with Bosma arhinia microphthalmia syndrome (Shaw et al. 2017), associated with CHH in 97% of subjects. This AD syndrome produces a severe highly penetrant disease. The zebrafish model showed a reduction of the projection length of the terminal nerve (where GnRH3 neurons are located) along with ethmoid plate abnormalities (Shaw et al. 2017).

## **Inheritance, oligogenicity and variants of uncertain significance**

All modes of transmission have been demonstrated in relation to the genetics of CHH involved, albeit with an ever-expanding role for oligogenicity since it was first reported (Pitteloud et al. 2007a, 2010; Sykiotis et al. 2010). Earlier publications in the field of CHH genetics (antecedent to the introduction of NGS techniques—NGS) necessarily lacked the capacity to identify potential deleterious variants in other loci, whether known or unknown. More recently, NGS is uncovering ever-increasing evidence of oligogenicity, at least partly explaining the apparent variable penetrance and expressivity of certain variants. Many heterozygous variants were previously believed to have a dominant (or incompletely penetrant) transmission, but we now know that only the most disrupting ones have a true AD inheritance and, in the vast majority of cases, CHH is more likely to arise from genetic hemizygoty (*ANOS1*), homozygoty, compound heterozygoty, or oligogenicity.

In this review we have reported more than sixty genes associated to CHH. However, no single study has ever

simultaneously evaluated this wide assortment of loci in a large patient cohort so as to provide a better understanding in a larger number of CHH patients. Knowledge of the oligogenic basis of CHH makes genotype–phenotype characterization increasingly uncertain, but it also explains the great variability found within and between kindreds that apparently share the same pathogenic genetic variant. The overlap between KS, nCHH, CPHD, AHH and HA further complicates the scenario making it even more essential to deploy NGS analyses using ever-wider panels of candidate genes (Raivio et al. 2012; Jayakody et al. 2012).

However, these advances in genomics come at a cost, with NGS allowing simultaneous screening of a vast number of genes, great numbers of variants of unknown clinical significance (VUS) are necessarily thrown up. The challenge now is to more cheaply, rapidly and reliably identify those variants that are truly pathogenic. Recent studies investigating individuals from general population as controls with NGS techniques have found 10–20% of them (according to the number of genes screened and the filtering criteria used to define a rare variant) harbouring heterozygous missense VUS in a CHH candidate gene (Sykiotis et al. 2010; Casatella et al. 2018; Cangiano et al. 2019), thereby reminding that not all the rare variants are able to disrupt the function of a protein. Therefore, many groups are implementing new methods to increase the specificity of genetic analyses so as to better distinguish pathogenic variants from among the VUS (Richards et al. 2015; Cox et al. 2018).

## **Genetic counselling**

With appropriate gonadotropin therapy and assisted reproductive techniques, many CHH men and women now have improved prospect of achieving fertility. In these individuals, genetic counselling forms an important component of the fertility management because of the potential risk of transmission to offspring. However, with the increasingly complex genetic architecture of CHH/KS propelled by massively parallel next-generation sequencing, providing informed, reliable and relevant genetic counselling has become incredibly challenging (Maione et al. 2018).

One of the significant challenges in predicting the risk of transmission of disease features is the difficulty in obtaining complete information on the phenotypes in all family members (ideally across three generations). It is further complicated by phenotypic expression that could be modified by different underlying genetic background (oligogenicity) as well as environmental exposure; variation in phenotype is frequently observed within the same family due to incomplete penetrance. Therefore, even for mutations with well-established classical Mendelian mode of transmission, variable expressivity and disease severity are likely.

Moreover, while oligogenicity has emerged to be an important genetic cause in a significant proportion of CHH subjects (Miraoui et al. 2013), distinguishing true oligogenicity from rare potentially deleterious variants that have yet to be equivocally linked to CHH remains a key challenge. Adding to the uncertainty is the different impact of individual mutations on disease manifestations by variants in other loci. It may also be impossible to pinpoint the likelihood of oligogenic transmission to offspring of affected parents in such cases because of missing information in respect of many of these putative genes.

On the other hand, knowledge of the specific genetic mutation could have important therapeutic implications. For instance, individuals with X-linked *NROB1* pathogenic variants tend to exhibit dismal response to gonadotropin therapy in the induction of spermatogenesis, with subsequent microsurgical sperm retrieval being required to have any hope of biological parenthood (Mantovani et al. 2006). These men should be counselled specifically, with alternative options such as donor sperm or adoption discussed early on.

Practically, for individuals harbouring mutations with classical Mendelian inheritance mode of transmission (AD, AR, or X-linked transmission), genetic counselling can be provided accordingly, along with other considerations as mentioned above. In cases of oligogenicity, a general estimate of 5–10% transmission risk may be counselled, while engaging them in continual discussion as novel information may arise from the constantly evolving genetic landscape from time to time (Sykiotis et al. 2010; Swee and Quinton 2019b). Importantly, recognizing that biochemical screening of offspring for minipuberty can be performed during the first 2–3 months of life, irrespective of whether the parental genotype is known, is a crucial early strategy in facilitating the exclusion of significant GnRH deficiency; it should be prudent to discuss this with potential future parents during the counselling process.

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## References

- Abreu AP, Trarbach EB, de Castro M et al (2008) Loss-of-function mutations in the genes encoding prokineticin-2 or prokineticin receptor-2 cause autosomal recessive Kallmann syndrome. *J Clin Endocrinol Metab* 93:4113–4118. <https://doi.org/10.1210/jc.2008-0958>
- Abreu AP, Noel SD, Xu S et al (2012) Evidence of the importance of the first intracellular loop of prokineticin receptor 2 in receptor function. *Mol Endocrinol* 26:1417–1427. <https://doi.org/10.1210/me.2012-1102>
- Alatzoglou KS, Kelberman D, Cowell CT et al (2011) Increased transactivation associated with SOX3 polyalanine tract deletion in a patient with hypopituitarism. *J Clin Endocrinol Metab*. <https://doi.org/10.1210/jc.2010-1239>
- Avilion AA, Nicolis SK, Pevny LH et al (2003) Multipotent cell lineages in early mouse development depend on SOX2 function. *Genes Dev* 17:126–140. <https://doi.org/10.1101/gad.224503>
- Ayari B, Soussi-Yanicostas N (2007) FGFR1 and anosmin-1 underlying genetically distinct forms of Kallmann syndrome are co-expressed and interact in olfactory bulbs. *Dev Genes Evol* 217:169–175. <https://doi.org/10.1007/s00427-006-0125-0>
- Bach I, Rhodes SJ, Pearce RV et al (1995) P-Lim, a LIM homeodomain factor, is expressed during pituitary organ and cell commitment and synergizes with Pit-1. *Proc Natl Acad Sci USA*. <https://doi.org/10.1073/pnas.92.7.2720>
- Balasubramanian R, Choi JH, Francescato L et al (2014) Functionally compromised CHD7 alleles in patients with isolated GnRH deficiency. *Proc Natl Acad Sci USA* 111:17953–17958. <https://doi.org/10.1073/pnas.1417438111>
- Balasubramanian R, Chew S, MacKinnon SE et al (2015) Expanding the phenotypic spectrum and variability of endocrine abnormalities associated with TUBB3 E410K syndrome. *J Clin Endocrinol Metab* 100:E473–E477. <https://doi.org/10.1210/jc.2014-4107>
- Bamshad M, Lin RC, Law DJ et al (1997) Mutations in human TBX3 alter limb, apocrine and genital development in ulnar-mammary syndrome. *Nat Genet* 16:311–315. <https://doi.org/10.1038/ng0797-311>
- Basaran Y, Bolu E, Unal HU et al (2013) Multiplex ligation dependent probe amplification analysis of KAL1, GNRH1, GNRHR, PROK2 and PROKR2 in male patients with idiopathic hypogonadotropic hypogonadism. *Endokrynol Pol* 64:285–292. <https://doi.org/10.5603/EP.2013.0007>
- Basciani S, Watanabe M, Mariani S et al (2012) Hypogonadism in a patient with two novel mutations of the luteinizing hormone  $\beta$ -subunit gene expressed in a compound heterozygous form. *J Clin Endocrinol Metab*. <https://doi.org/10.1210/jc.2012-1986>
- Bencharit S, Bin CC, Siddiqui A et al (2007) Structural insights into fibronectin type III domain-mediated signaling. *J Mol Biol* 367:303–309. <https://doi.org/10.1016/j.jmb.2006.10.017>
- Beranova M, Oliveira LM, Bédécarrats GY et al (2001) Prevalence, phenotypic spectrum, and modes of inheritance of gonadotropin-releasing hormone receptor mutations in idiopathic hypogonadotropic hypogonadism. *J Clin Endocrinol Metab* 86:1580–1588. <https://doi.org/10.1210/jcem.86.4.7395>
- Bernard G, Chouery E, Putorti ML et al (2011) Mutations of POLR3A encoding a catalytic subunit of RNA polymerase Pol III cause a recessive hypomyelinating leukodystrophy. *Am J Hum Genet* 89:415–423. <https://doi.org/10.1016/j.ajhg.2011.07.014>
- Bhagavath B, Podolsky RH, Ozata M et al (2006) Clinical and molecular characterization of a large sample of patients with hypogonadotropic hypogonadism. *Fertil Steril* 85:706–713. <https://doi.org/10.1016/j.fertnstert.2005.08.044>
- Bhangoo APS, Hunter CS, Savage JJ et al (2006) A novel LHX3 mutation presenting as combined pituitary hormonal deficiency. *J Clin Endocrinol Metab* 91(3):747–753. <https://doi.org/10.1210/jc.2005-2360>
- Bianco SDC, Kaiser UB (2009) The genetic and molecular basis of idiopathic hypogonadotropic hypogonadism. *Nat Rev Endocrinol* 5:569–576. <https://doi.org/10.1038/nrendo.2009.177>
- Blewitt ME, Gendrel A-V, Pang Z et al (2008) SmcHD1, containing a structural-maintenance-of-chromosomes hinge domain, has a critical role in X inactivation. *Nat Genet* 40:663–669. <https://doi.org/10.1038/ng.142>
- Boehm U, Bouloux P-M, Dattani MT et al (2015) European Consensus Statement on congenital hypogonadotropic

- hypogonadism—pathogenesis, diagnosis and treatment. *Nat Rev Endocrinol* 11:547–564. <https://doi.org/10.1038/nrendo.2015.112>
- Bonfig W, Krude H, Schmidt H (2011) A novel mutation of LHX3 is associated with combined pituitary hormone deficiency including ACTH deficiency, sensorineural hearing loss, and short neck—a case report and review of the literature. *Eur J Pediatr*. <https://doi.org/10.1007/s00431-011-1393-x>
- Bonomi M, Libri DV, Guizzardi F et al (2012) New understandings of the genetic basis of isolated idiopathic central hypogonadism. *Asian J Androl* 14:49–56. <https://doi.org/10.1038/aja.2011.68>
- Bonomi M, Vezzoli V, Krausz C et al (2018) Characteristics of a nationwide cohort of patients presenting with isolated hypogonadotropic hypogonadism (IHH). *Eur J Endocrinol* 178:23–32. <https://doi.org/10.1530/EJE-17-0065>
- Böttcher RT, Pollet N, Delius H, Niehrs C (2004) The transmembrane protein XFLRT3 forms a complex with FGF receptors and promotes FGF signalling. *Nat Cell Biol*. <https://doi.org/10.1038/ncb1082>
- Bouilly J, Messina A, Papadakis G et al (2018) DCC/NTN1 complex mutations in patients with congenital hypogonadotropic hypogonadism impair GnRH neuron development. *Hum Mol Genet* 27:359–372. <https://doi.org/10.1093/hmg/ddx408>
- Bouligand J, Ghervan C, Tello JA et al (2009) Isolated familial hypogonadotropic hypogonadism and a GNRH1 mutation. *N Engl J Med* 360:2742–2748. <https://doi.org/10.1056/NEJMoa0900136>
- Breuskin I, Bodson M, Thelen N et al (2009) Sox10 promotes the survival of cochlear progenitors during the establishment of the organ of Corti. *Dev Biol*. <https://doi.org/10.1016/j.ydbio.2009.09.007>
- Brioude F, Bouligand J, Trabado S et al (2010) Non-syndromic congenital hypogonadotropic hypogonadism: clinical presentation and genotype-phenotype relationships. *Eur J Endocrinol* 162:835–851. <https://doi.org/10.1530/EJE-10-0083>
- Bülow HE, Berry KL, Topper LH et al (2002) Heparan sulfate proteoglycan-dependent induction of axon branching and axon misrouting by the Kallmann syndrome gene kal-1. *Proc Natl Acad Sci USA* 99:6346–6351. <https://doi.org/10.1073/pnas.092128099>
- Cangiano B, Duminuco P, Vezzoli V et al (2019) Evidence for a common genetic origin of classic and milder adult-onset forms of isolated hypogonadotropic hypogonadism. *J Clin Med*. <https://doi.org/10.3390/jcm8010126>
- Cariboni A, Pimpinelli F, Colamarino S et al (2004) The product of X-linked Kallmann's syndrome gene (KAL1) affects the migratory activity of gonadotropin-releasing hormone (GnRH)-producing neurons. *Hum Mol Genet* 13:2781–2791. <https://doi.org/10.1093/hmg/ddh309>
- Cariboni A, Davidson K, Rakic S et al (2011) Defective gonadotropin-releasing hormone neuron migration in mice lacking SEMA3A signalling through NR1 and NRP2: implications for the aetiology of hypogonadotropic hypogonadism. *Hum Mol Genet* 20:336–344. <https://doi.org/10.1093/hmg/ddq468>
- Cariboni A, André V, Chauvet S et al (2015) Dysfunctional SEMA3E signaling underlies gonadotropin-releasing hormone neuron deficiency in Kallmann syndrome. *J Clin Invest* 125:2413–2428. <https://doi.org/10.1172/JCI78448>
- Caronia LM, Martin C, Welt CK et al (2011) A genetic basis for functional hypothalamic amenorrhea. *N Engl J Med* 364:215–225. <https://doi.org/10.1056/NEJMoa0911064>
- Casoni F, Malone SA, Belle M et al (2016) Development of the neurons controlling fertility in humans: new insights from 3D imaging and transparent fetal brains. *Development* 143:3969–3981. <https://doi.org/10.1242/dev.139444>
- Cassatella D, Howard SR, Acierio JS et al (2018) Congenital hypogonadotropic hypogonadism and constitutional delay of growth and puberty have distinct genetic architectures. *Eur J Endocrinol* 178:377–388. <https://doi.org/10.1530/EJE-17-0568>
- Cattanach BM, Iddon CA, Charlton HM et al (1977) Gonadotropin-releasing hormone deficiency in a mutant mouse with hypogonadism. *Nature* 269:338–340. <https://doi.org/10.1038/269338a0>
- Cavalcante JC, Bittencourt JC, Elias CF (2014) Distribution of the neuronal inputs to the ventral premammillary nucleus of male and female rats. *Brain Res* 1582:77–90. <https://doi.org/10.1016/j.brainres.2014.07.034>
- Cerrato F, Shagoury J, Kralickova M et al (2006) Coding sequence analysis of GNRHR and GPR54 in patients with congenital and adult-onset forms of hypogonadotropic hypogonadism. *Eur J Endocrinol* 155(Suppl):S3–S10. <https://doi.org/10.1530/eje.1.02235>
- Ceylan AC, Gursoy H, Yildirim N et al (2017) Clinical heterogeneity associated with TUBB3 gene mutation in a Turkish family with congenital fibrosis of the extraocular muscles. *Ophthalmic Genet* 38:288–290. <https://doi.org/10.1080/13816810.2016.1193881>
- Chan Y-M, de Guillebon A, Lang-Muritano M et al (2009) GNRH1 mutations in patients with idiopathic hypogonadotropic hypogonadism. *Proc Natl Acad Sci USA* 106:11703–11708. <https://doi.org/10.1073/pnas.0903449106>
- Charles MA, Saunders TL, Wood WM et al (2006) Pituitary-specific Gata2 knockout: effects on gonadotrope and thyrotrope function. *Mol Endocrinol*. <https://doi.org/10.1210/me.2005-0378>
- Chew S, Balasubramanian R, Chan W-M et al (2013) A novel syndrome caused by the E410K amino acid substitution in the neuronal  $\beta$ -tubulin isotype 3. *Brain* 136:522–535. <https://doi.org/10.1093/brain/aws345>
- Chung WCJ, Tsai P-S (2010) Role of fibroblast growth factor signalling in gonadotropin-releasing hormone neuronal system development. Kallmann syndrome and hypogonadotropic hypogonadism. *KARGER, Basel*, pp 37–50
- Chung WCJ, Matthews TA, Tata BK, Tsai PS (2010) Compound deficiencies in multiple fibroblast growth factor signalling components differentially impact the murine gonadotropin-releasing hormone system. *J Neuroendocrinol*. <https://doi.org/10.1111/j.1365-2826.2010.02024.x>
- Cimino I, Casoni F, Liu X et al (2016) Novel role for anti-Müllerian hormone in the regulation of GnRH neuron excitability and hormone secretion. *Nat Commun* 7:10055. <https://doi.org/10.1038/ncomms10055>
- Cioppi F, Riera-Escamilla A, Manilall A et al (2019) Genetics of ncHH: from a peculiar inheritance of a novel GNRHR mutation to a comprehensive review of the literature. *Andrology* 7:88–101. <https://doi.org/10.1111/andr.12563>
- Clément K, Vaisse C, Lahlou N et al (1998) A mutation in the human leptin receptor gene causes obesity and pituitary dysfunction. *Nature*. <https://doi.org/10.1038/32911>
- Cole LW, Sidis Y, Zhang C et al (2008) Mutations in prokineticin 2 and prokineticin receptor 2 genes in human gonadotropin-releasing hormone deficiency: molecular genetics and clinical spectrum. *J Clin Endocrinol Metab* 93:3551–3559. <https://doi.org/10.1210/jc.2007-2654>
- Costa EMF, Bedecarrats GY, Mendonca BB et al (2001) Two novel mutations in the gonadotropin-releasing hormone receptor gene in Brazilian patients with hypogonadotropic hypogonadism and normal olfaction<sup>1</sup>. *J Clin Endocrinol Metab* 86:2680–2686. <https://doi.org/10.1210/jcem.86.6.7551>
- Costa-Barbosa FA, Balasubramanian R, Keefe KW et al (2013) Prioritizing genetic testing in patients with Kallmann syndrome using clinical phenotypes. *J Clin Endocrinol Metab* 98:E943–E953. <https://doi.org/10.1210/jc.2012-4116>
- Cox KH, Oliveira LMB, Plummer L et al (2018) Modeling mutant/wild-type interactions to ascertain pathogenicity of PROKR2 missense variants in patients with isolated GnRH deficiency.

- Hum Mol Genet 27:338–350. <https://doi.org/10.1093/hmg/ddx404>
- Cravo RM, Frazao R, Perello M et al (2013) Leptin signaling in Kiss1 neurons arises after pubertal development. *PLoS ONE* 8:e58698. <https://doi.org/10.1371/journal.pone.0058698>
- Dai W, Wu J, Zhao Y et al (2019) Functional analysis of SOX10 mutations identified in Chinese patients with Kallmann syndrome. *Gene* 702:99–106. <https://doi.org/10.1016/j.gene.2019.03.039>
- Daoud H, Tétreault M, Gibson W et al (2013) Mutations in POLR3A and POLR3B are a major cause of hypomyelinating leukodystrophies with or without dental abnormalities and/or hypogonadotropic hypogonadism. *J Med Genet* 50:194–197. <https://doi.org/10.1136/jmedgenet-2012-101357>
- Dasen JS, Rosenfeld MG (2001) Signaling and transcriptional mechanisms in pituitary development. *Annu Rev Neurosci* 24:327–355. <https://doi.org/10.1146/annurev.neuro.24.1.327>
- Dattani MT, Robinson IC (2000) The molecular basis for developmental disorders of the pituitary gland in man. *Clin Genet* 57(5):337–346. <https://doi.org/10.1034/j.1399-0004.2000.570503.x>
- Dattani MT, Martinez-Barbera JP, Thomas PQ et al (1998) Mutations in the homeobox gene HESX1/Hesx1 associated with septo-optic dysplasia in human and mouse. *Nat Genet*. <https://doi.org/10.1038/477>
- Diazok D, Divall S, Matsuo I et al (2011) Deletion of Otx2 in GnRH neurons results in a mouse model of hypogonadotropic hypogonadism. *Mol Endocrinol*. <https://doi.org/10.1210/me.2010-0271>
- Dodé C, Hardelin J-P (2009) Kallmann syndrome. *Eur J Hum Genet* 17:139–146. <https://doi.org/10.1038/ejhg.2008.206>
- Dodé C, Levilliers J, Dupont JM et al (2003) Loss-of-function mutations in FGFR1 cause autosomal dominant Kallmann syndrome. *Nat Genet*. <https://doi.org/10.1038/ng1122>
- Dodé C, Teixeira L, Levilliers J et al (2006) Kallmann syndrome: mutations in the genes encoding prokineticin-2 and prokineticin receptor-2. *PLoS Genet* 2:e175. <https://doi.org/10.1371/journal.pgen.0020175>
- Duan J, Chen R-R, Li L et al (2014) Reversal of idiopathic hypogonadotropic hypogonadism: a cohort study in Chinese patients. *Asian J Androl*. <https://doi.org/10.4103/1008-682X.145072>
- Duke VM, Winyard PJD, Thorogood P et al (1995) KAL, a gene mutated in Kallmann's syndrome, is expressed in the first trimester of human development. *Mol Cell Endocrinol* 110:73–79. [https://doi.org/10.1016/0303-7207\(95\)03518-C](https://doi.org/10.1016/0303-7207(95)03518-C)
- Dwyer AA, Hayes FJ, Plummer L et al (2010) The long-term clinical follow-up and natural history of men with adult-onset idiopathic hypogonadotropic hypogonadism. *J Clin Endocrinol Metab* 95:4235–4243. <https://doi.org/10.1210/jc.2010-0245>
- Dwyer AA, Chavan NR, Lewkowicz-Shpuntoff H et al (2019a) Functional hypogonadotropic hypogonadism in men: underlying neuroendocrine mechanisms and natural history. *J Clin Endocrinol Metab* 104:3403–3414. <https://doi.org/10.1210/jc.2018-02697>
- Dwyer AA, Smith N, Quinton R (2019b) Psychological aspects of congenital hypogonadotropic hypogonadism. *Front Endocrinol (Lausanne)* 10:353. <https://doi.org/10.3389/fendo.2019.00353>
- Dzemaili S, Tiemensma J, Quinton R et al (2017) Beyond hormone replacement: quality of life in women with congenital hypogonadotropic hypogonadism. *Endocr Connect* 6:404–412. <https://doi.org/10.1530/EC-17-0095>
- Falardeau J, Chung WCJ, Beenken A et al (2008) Decreased FGF8 signaling causes deficiency of gonadotropin-releasing hormone in humans and mice. *J Clin Invest* 118:2822–2831. <https://doi.org/10.1172/JCI34538>
- Fang Q, Benedetti AFF, Ma Q et al (2016a) HESX1 mutations in patients with congenital hypopituitarism: variable phenotypes with the same genotype. *Clin Endocrinol (Oxf)*. <https://doi.org/10.1111/cen.13067>
- Fang Q, George AS, Brinkmeier ML et al (2016) Genetics of combined pituitary hormone deficiency: roadmap into the genome era. *Endocr Rev* 37(6):636–675. <https://doi.org/10.1210/er.2016-1101>
- Fantes J, Ragge NK, Lynch S-A et al (2003) Mutations in SOX2 cause anophthalmia. *Nat Genet* 33:461–463. <https://doi.org/10.1038/ng1120>
- Farooqi IS (2002) Leptin and the onset of puberty: insights from rodent and human genetics. *Semin Reprod Med*. <https://doi.org/10.1055/s-2002-32505>
- Farooqi IS, Jebb SA, Langmack G et al (1999) Effects of recombinant leptin therapy in a child with congenital leptin deficiency. *N Engl J Med*. <https://doi.org/10.1056/NEJM199909163411204>
- Farooqi IS, Volders K, Stanhope R et al (2007a) Hyperphagia and early-onset obesity due to a novel homozygous missense mutation in prohormone convertase 1/3. *J Clin Endocrinol Metab* 92(9):3369–3373. <https://doi.org/10.1210/jc.2007-0687>
- Farooqi IS, Wangenstein T, Collins S et al (2007b) Clinical and molecular genetic spectrum of congenital deficiency of the leptin receptor. *N Engl J Med*. <https://doi.org/10.1056/NEJMoa063988>
- Franco B, Guioli S, Pragliola A et al (1991) A gene deleted in Kallmann's syndrome shares homology with neural cell adhesion and axonal path-finding molecules. *Nature* 353:529–536. <https://doi.org/10.1038/353529a0>
- Francou B, Bouligand J, Voican A et al (2011) Normosmic congenital hypogonadotropic hypogonadism due to TAC3/TACR3 mutations: characterization of neuroendocrine phenotypes and novel mutations. *PLoS ONE* 6:e25614. <https://doi.org/10.1371/journal.pone.0025614>
- Francou B, Paul C, Amazit L et al (2016) Prevalence of *KISS1 Receptor* mutations in a series of 603 patients with normosmic congenital hypogonadotropic hypogonadism and characterization of novel mutations: a single-centre study. *Hum Reprod* 31:1363–1374. <https://doi.org/10.1093/humrep/dew073>
- Fromantin M, Gineste J, Didier A, Rouvier J (1973) Impuberism and hypogonadism at induction into military service. Statistical study. *Probl Actuels Endocrinol Nutr* 16:179–199
- Funes S, Hedrick JA, Vassileva G et al (2003) The KiSS-1 receptor GPR54 is essential for the development of the murine reproductive system. *Biochem Biophys Res Commun* 312:1357–1363. <https://doi.org/10.1016/j.bbrc.2003.11.066>
- Galazzi E, Duminuco P, Moro M et al (2018) Hypogonadotropic hypogonadism and pituitary hypoplasia as recurrent features in Ulnar-Mammary syndrome. *Endocr Connect* 7:1432–1441. <https://doi.org/10.1530/EC-18-0486>
- Gianetti E, Tusset C, Noel SD et al (2010) TAC3/TACR3 mutations reveal preferential activation of gonadotropin-releasing hormone release by neurokinin B in neonatal life followed by reversal in adulthood. *J Clin Endocrinol Metab* 95:2857–2867. <https://doi.org/10.1210/jc.2009-2320>
- Gonçalves CI, Aragüés JM, Bastos M et al (2017) GNRHR biallelic and digenic mutations in patients with normosmic congenital hypogonadotropic hypogonadism. *Endocr Connect* 6:360–366. <https://doi.org/10.1530/EC-17-0104>
- Gonçalves CI, Patriarca FM, Aragüés JM et al (2019) High frequency of CHD7 mutations in congenital hypogonadotropic hypogonadism. *Sci Rep* 9:1597. <https://doi.org/10.1038/s41598-018-38178-y>
- Gonzalez-Martinez D (2004) Anosmin-1 modulates fibroblast growth factor receptor 1 signaling in human gonadotropin-releasing hormone olfactory neuroblasts through a heparan sulfate-dependent mechanism. *J Neurosci* 24:10384–10392. <https://doi.org/10.1523/JNEUROSCI.3400-04.2004>
- Goodman RL, Lehman MN, Smith JT et al (2007) Kisspeptin neurons in the arcuate nucleus of the ewe express both dynorphin A and neurokinin B. *Endocrinology* 148:5752–5760. <https://doi.org/10.1210/en.2007-0961>

- Gorbenko Del Blanco D, Romero CJ, Diaczok D et al (2012) A novel OTX2 mutation in a patient with combined pituitary hormone deficiency, pituitary malformation, and an underdeveloped left optic nerve. *Eur J Endocrinol*. <https://doi.org/10.1530/EJE-12-0333>
- Gottsch ML, Cunningham MJ, Smith JT et al (2004) A role for kisspeptins in the regulation of gonadotropin secretion in the mouse. *Endocrinology* 145:4073–4077. <https://doi.org/10.1210/en.2004-0431>
- Habiby RL, Boepple P, Nachtigall L et al (1996) Adrenal hypoplasia congenita with hypogonadotropic hypogonadism: evidence that DAX-1 mutations lead to combined hypothalamic and pituitary defects in gonadotropin production. *J Clin Invest* 98:1055–1062. <https://doi.org/10.1172/JCI118866>
- Haines DE, Mihailoff GA, Parent AD, Perkins E (2018) The hypothalamus. *Fundam Neurosci Basic Clin Appl* 442–456:e1. <https://doi.org/10.1016/B978-0-323-39632-5.00030-X>
- Hanafusa H, Torii S, Yasunaga T, Nishida E (2002) Sprouty1 and Sprouty2 provide a control mechanism for the Ras/MAPK signalling pathway. *Nat Cell Biol* 4:850–858. <https://doi.org/10.1038/ncb867>
- Hanchate NK, Giacobini P, Lhuillier P et al (2012) SEMA3A, a gene involved in axonal pathfinding, is mutated in patients with kallmann syndrome. *PLoS Genet* 8:e1002896. <https://doi.org/10.1371/journal.pgen.1002896>
- Hausott B, Vallant N, Schlick B et al (2012) Sprouty2 and -4 regulate axon outgrowth by hippocampal neurons. *Hippocampus*. <https://doi.org/10.1002/hipo.20910>
- Henderson RH, Williamson KA, Kennedy JS et al (2009) A rare de novo nonsense mutation in OTX2 causes early onset retinal dystrophy and pituitary dysfunction. *Mol Vis* 15:2442–2447
- Hirata T, Nakazawa M, Yoshihara S et al (2006) Zinc-finger gene Fez in the olfactory sensory neurons regulates development of the olfactory bulb non-cell-autonomously. *Development* 133:1433–1443. <https://doi.org/10.1242/dev.02329>
- Hoffmann HM, Pandolfi EC, Larder R, Mellon PL (2019) Haploinsufficiency of homeodomain proteins Six3, Vax1, and Otx2 causes subfertility in mice via distinct mechanisms. *Neuroendocrinology*. <https://doi.org/10.1159/000494086>
- Howard SR, Guasti L, Ruiz-Babot G et al (2016) IGSF 10 mutations dysregulate gonadotropin-releasing hormone neuronal migration resulting in delayed puberty. *EMBO Mol Med* 8:626–642. <https://doi.org/10.15252/emmm.201606250>
- Howard SR, Oleari R, Poliandri A et al (2018) HS6ST1 insufficiency causes self-limited delayed puberty in contrast with other GnRH deficiency genes. *J Clin Endocrinol Metab* 103:3420–3429. <https://doi.org/10.1210/jc.2018-00646>
- Huang H, Yang T, Shao Q et al (2018) Human TUBB3 mutations disrupt netrin attractive signaling. *Neuroscience* 374:155–171. <https://doi.org/10.1016/j.neuroscience.2018.01.046>
- Hudson R, Laska M, Berger T et al (1994) Olfactory function in patients with hypogonadotropic hypogonadism: an all-or-none phenomenon? *Chem Senses* 19:57–69. <https://doi.org/10.1093/chemse/19.1.57>
- Hutchins BI, Kotan LD, Taylor-Burds C et al (2016) CCDC141 mutation identified in anosmic hypogonadotropic hypogonadism (Kallmann Syndrome) alters GnRH neuronal migration. *Endocrinology* 157:1956–1966. <https://doi.org/10.1210/en.2015-1846>
- Iacovazzo D, Carlsen E, Lugli F et al (2016) Factors predicting pasireotide responsiveness in somatotroph pituitary adenomas resistant to first-generation somatostatin analogues: an immunohistochemical study. *Eur J Endocrinol* 174:241–250. <https://doi.org/10.1530/EJE-15-0832>
- Izumi Y, Suzuki E, Kanzaki S et al (2014) Genome-wide copy number analysis and systematic mutation screening in 58 patients with hypogonadotropic hypogonadism. *Fertil Steril*. <https://doi.org/10.1016/j.fertnstert.2014.06.017>
- Jackson RS, Creemers JWM, Ohagi S et al (1997) Obesity and impaired prohormone processing associated with mutations in the human prohormone convertase 1 gene. *Nat Genet*. <https://doi.org/10.1038/ng0797-303>
- Jarzabek K, Wolczynski S, Lesniewicz R et al (2012) Evidence that FGFR1 loss-of-function mutations may cause variable skeletal malformations in patients with Kallmann syndrome. *Adv Med Sci*. <https://doi.org/10.2478/v10039-012-0036-4>
- Jayakody SA, Andoniadou CL, Gaston-Massuet C et al (2012) SOX2 regulates the hypothalamic-pituitary axis at multiple levels. *J Clin Invest* 122:3635–3646. <https://doi.org/10.1172/JCI64311>
- Jongmans MCJ, Admiraal RJ, Van Der Donk KP et al (2006) CHARGE syndrome: the phenotypic spectrum of mutations in the CHD7 gene. *J Med Genet*. <https://doi.org/10.1136/jmg.2005.036061>
- Jongmans MCJ, van Ravenswaaij-Arts CMA, Pitteloud N et al (2009) CHD7 mutations in patients initially diagnosed with Kallmann syndrome—the clinical overlap with CHARGE syndrome. *Clin Genet*. <https://doi.org/10.1111/j.1399-0004.2008.01107.x>
- Känsäkoski J, Fagerholm R, Laitinen EM et al (2014) Mutation screening of SEMA3A and SEMA7A in patients with congenital hypogonadotropic hypogonadism. *Pediatr Res* 75:641–644. <https://doi.org/10.1038/pr.2014.23>
- Kawamata N, Sakajiri S, Sugimoto K-J et al (2002) A novel chromosomal translocation t(1;14)(q25;q32) in pre-B acute lymphoblastic leukemia involves the LIM homeodomain protein gene, Lhx4. *Oncogene* 21:4983–4991. <https://doi.org/10.1038/sj.onc.1205628>
- Kelberman D, Rizzoti K, Avilion A et al (2006) Mutations within Sox2/SOX2 are associated with abnormalities in the hypothalamo-pituitary-gonadal axis in mice and humans.e. *J Clin Invest* 116:2442–2455. <https://doi.org/10.1172/JCI28658>
- Kelberman D, Turton JPG, Woods KS et al (2009) Molecular analysis of novel PROP1 mutations associated with combined pituitary hormone deficiency (CPHD). *Clin Endocrinol (Oxf)*. <https://doi.org/10.1111/j.1365-2265.2008.03326.x>
- Kevelam SHG, Van Harssel JJT, Van Der Zwaag B et al (2012) A patient with a mild holoprosencephaly spectrum phenotype and heterotaxy and a 1.3 Mb deletion encompassing GLI2. *Am J Med Genet Part A*. <https://doi.org/10.1002/ajmg.a.34350>
- Kim H-G, Layman LC (2011) The role of CHD7 and the newly identified WDR11 gene in patients with idiopathic hypogonadotropic hypogonadism and Kallmann syndrome. *Mol Cell Endocrinol* 346:74–83. <https://doi.org/10.1016/j.mce.2011.07.013>
- Kim HG, Kurth I, Lan F et al (2008a) Mutations in CHD7, encoding a chromatin-remodeling protein, cause idiopathic hypogonadotropic hypogonadism and Kallmann syndrome. *Am J Hum Genet*. <https://doi.org/10.1016/j.ajhg.2008.09.005>
- Kim S-H, Hu Y, Cadman S, Bouloux P (2008b) Diversity in fibroblast growth factor receptor 1 regulation: learning from the investigation of Kallmann syndrome. *J Neuroendocrinol* 20:141–163. <https://doi.org/10.1111/j.1365-2826.2007.01627.x>
- Kim H-G, Ahn J-W, Kurth I et al (2010) WDR11, a WD protein that interacts with transcription factor EMX1, is mutated in idiopathic hypogonadotropic hypogonadism and Kallmann syndrome. *Am J Hum Genet* 87:465–479. <https://doi.org/10.1016/j.ajhg.2010.08.018>
- Kotan LD, Hutchins BI, Ozkan Y et al (2014) Mutations in FEZF1 cause Kallmann syndrome. *Am J Hum Genet* 95:326–331. <https://doi.org/10.1016/j.ajhg.2014.08.006>
- Kramer PR, Wray S (2000) Novel gene expressed in nasal region influences outgrowth of olfactory axons and migration of luteinizing hormone-releasing hormone (LHRH) neurons. *Genes Dev* 14:1824–1834

- Kramer PR, Wray S (2001) Nasal embryonic LHRH factor (NELF) expression within the CNS and PNS of the rodent. *Brain Res Gene Expr Patterns* 1:23–26
- Kuang X-L, Zhao X-M, Xu H-F et al (2010) Spatio-temporal expression of a novel neuron-derived neurotrophic factor (NDNF) in mouse brains during development. *BMC Neurosci* 11:137. <https://doi.org/10.1186/1471-2202-11-137>
- Kurokawa D, Kiyonari H, Nakayama R et al (2004) Regulation of Otx2 expression and its functions in mouse forebrain and midbrain. *Development*. <https://doi.org/10.1242/dev.01220>
- Kyriakakis N, Shonibare T, Kyaw-Tun J et al (2017) Late-onset X-linked adrenal hypoplasia (DAX-1, NR0B1): two new adult-onset cases from a single center. *Pituitary*. <https://doi.org/10.1007/s11102-017-0822-x>
- Laitinen E-M, Vaaralahti K, Tommiska J et al (2011) Incidence, phenotypic features and molecular genetics of Kallmann syndrome in Finland. *Orphanet J Rare Dis* 6:41. <https://doi.org/10.1186/1750-1172-6-41>
- Lakhina V, Marcaccio CL, Shao X et al (2012) Netrin/DCC signaling guides olfactory sensory axons to their correct location in the olfactory bulb. *J Neurosci* 32:4440–4456. <https://doi.org/10.1523/JNEUROSCI.4442-11.2012>
- Lalani SR, Safiullah AM, Molinari LM et al (2004) SEMA3E mutation in a patient with CHARGE syndrome. *J Med Genet* 41:e94. <https://doi.org/10.1136/jmg.2003.017640>
- Layman LC, Lee EJ, Peak DB et al (1997) Delayed puberty and hypogonadism caused by mutations in the follicle-stimulating hormone  $\beta$ -subunit gene. *N Engl J Med*. <https://doi.org/10.1056/NEJM199708283370905>
- Layman LC, Cohen DP, Jin M et al (1998) Mutations in gonadotropin-releasing hormone receptor gene cause hypogonadotropic hypogonadism. *Nat Genet* 18:14–15. <https://doi.org/10.1038/ng0198-14>
- Layman LC, Porto ALA, Xie J et al (2002) FSH $\beta$  gene mutations in a female with partial breast development and a male sibling with normal puberty and azoospermia. *J Clin Endocrinol Metab*. <https://doi.org/10.1210/jc.87.8.3702>
- Lee GH, Proenca R, Montez JM et al (1996) Abnormal splicing of the leptin receptor in diabetic mice. *Nature*. <https://doi.org/10.1038/379632a0>
- Legouis R, Hardelin JP, Leveilliers J et al (1991) The candidate gene for the X-linked Kallmann syndrome encodes a protein related to adhesion molecules. *Cell* 67:423–435. [https://doi.org/10.1016/0092-8674\(91\)90193-3](https://doi.org/10.1016/0092-8674(91)90193-3)
- Leroy C, Fouveaut C, Leclercq S et al (2008) Biallelic mutations in the prokineticin-2 gene in two sporadic cases of Kallmann syndrome. *Eur J Hum Genet* 16:865–868. <https://doi.org/10.1038/ejhg.2008.15>
- Lewkowitz-Shpuntoff HM, Hughes VA, Plummer L et al (2012) Olfactory phenotypic spectrum in idiopathic hypogonadotropic hypogonadism: pathophysiological and genetic implications. *J Clin Endocrinol Metab* 97:E136–E144. <https://doi.org/10.1210/jc.2011-2041>
- Li C, Scott DA, Hatch E et al (2007) Dusp6 (Mkp3) is a negative feedback regulator of Fgf-stimulated ERK signaling during mouse development. *Development*. <https://doi.org/10.1242/dev.02701>
- Libri DV, Kleinau G, Vezzoli V et al (2014) Germline prokineticin receptor 2 (PROKR2) variants associated with central hypogonadism cause differential modulation of distinct intracellular pathways. *J Clin Endocrinol Metab* 99:E458–E463. <https://doi.org/10.1210/jc.2013-2431>
- Liu G, Beggs H, Jürgensen C et al (2004) Netrin requires focal adhesion kinase and Src family kinases for axon outgrowth and attraction. *Nat Neurosci* 7:1222–1232. <https://doi.org/10.1038/nn1331>
- Lo A, Zheng W, Gong Y et al (2011) GATA transcription factors regulate LH $\beta$  gene expression. *J Mol Endocrinol* 47:45–58. <https://doi.org/10.1530/JME-10-0137>
- Lofrano-Porto A, Barra GB, Giacomini LA et al (2007) Luteinizing hormone beta mutation and hypogonadism in men and women. *N Engl J Med*. <https://doi.org/10.1056/NEJMoa071999>
- Machinis K, Pantel J, Netchine I et al (2001) Syndromic short stature in patients with a germline mutation in the LIM homeobox LHX4. *Am J Hum Genet* 69:961–968. <https://doi.org/10.1086/323764>
- Maione L, Brailly-Tabard S, Nevoux J et al (2016) Reversal of congenital hypogonadotropic hypogonadism in a man with Kallmann syndrome due to *SOX10* mutation. *Clin Endocrinol (Oxf)* 85:988–989. <https://doi.org/10.1111/cen.13231>
- Maione L, Dwyer AA, Francou B et al (2018) Genetic counseling for congenital hypogonadotropic hypogonadism and Kallmann syndrome: new challenges in the era of oligogenism and next-generation sequencing. *Eur J Endocrinol* 178(3):R55–R80 <https://doi.org/10.1530/EJE-17-0749>
- Malone SA, Papadakis GE, Messina A et al (2019) Defective AMH signaling disrupts GnRH neuron development and function and contributes to hypogonadotropic hypogonadism. *Elife*. <https://doi.org/10.7554/eLife.47198>
- Mantovani G, De Menis E, Borretta G et al (2006) DAX1 and X-linked adrenal hypoplasia congenita: clinical and molecular analysis in five patients. *Eur J Endocrinol*. <https://doi.org/10.1530/eje.1.02132>
- Marcos S, Monnier C, Rovira X et al (2017) Defective signaling through plexin-A1 compromises the development of the peripheral olfactory system and neuroendocrine reproductive axis in mice. *Hum Mol Genet* 26:2006–2017. <https://doi.org/10.1093/hmg/ddx080>
- Margolin DH, Kousi M, Chan Y-M et al (2013) Ataxia, dementia, and hypogonadotropism caused by disordered ubiquitination. *N Engl J Med* 368:1992–2003. <https://doi.org/10.1056/NEJMoa1215993>
- Marsh APL, Heron D, Edwards TJ et al (2017) Mutations in DCC cause isolated agenesis of the corpus callosum with incomplete penetrance. *Nat Genet* 49:511–514. <https://doi.org/10.1038/ng.3794>
- Matsumoto S-I, Yamazaki C, Masumoto K-H et al (2006) Abnormal development of the olfactory bulb and reproductive system in mice lacking prokineticin receptor PKR2. *Proc Natl Acad Sci USA* 103:4140–4145. <https://doi.org/10.1073/pnas.0508881103>
- McCabe MJ, Gaston-Massuet C, Tziaferi V et al (2011a) Novel FGF8 mutations associated with recessive holoprosencephaly, craniofacial defects, and hypothalamo-pituitary dysfunction. *J Clin Endocrinol Metab*. <https://doi.org/10.1210/jc.2011-0454>
- McCabe MJ, Gaston-Massuet C, Gregory LC et al (2013) Variations in PROKR2, but not PROK2, are associated with hypopituitarism and septo-optic dysplasia. *J Clin Endocrinol Metab* 98:E547–E557. <https://doi.org/10.1210/jc.2012-3067>
- McCabe MJ, Hu Y, Gregory LC et al (2015) Novel application of luciferase assay for the in vitro functional assessment of KAL1 variants in three females with septo-optic dysplasia (SOD). *Mol Cell Endocrinol* 417:63–72. <https://doi.org/10.1016/j.mce.2015.09.010>
- McCormack SE, Li D, Kim YJ et al (2017) Digenic inheritance of PROKR2 and WDR11 mutations in pituitary stalk interruption syndrome. *J Clin Endocrinol Metab* 102:2501–2507. <https://doi.org/10.1210/jc.2017-00332>
- McNay DEG, Turton JP, Kelberman D et al (2007) HESX1 mutations are an uncommon cause of septo-optic dysplasia and hypopituitarism. *J Clin Endocrinol Metab*. <https://doi.org/10.1210/jc.2006-1609>
- Men M, Wu J, Zhao Y et al (2019) Genotypic and phenotypic spectra of FGFR1, FGF8, and FGF17 mutations in a Chinese cohort with idiopathic hypogonadotropic hypogonadism. *Fertil Steril*. <https://doi.org/10.1016/j.fertnstert.2019.08.069>

- Mengen E, Tunc S, Kotan LD et al (2016) Complete idiopathic hypogonadotropic hypogonadism due to homozygous GNRH1 mutations in the mutational hot spots in the region encoding the decapeptide. *Horm Res Paediatr* 85:107–111. <https://doi.org/10.1159/000441977>
- Messina A, Giacobini P (2013) Semaphorin signaling in the development and function of the gonadotropin hormone-releasing hormone system. *Front Endocrinol (Lausanne)* 4:133. <https://doi.org/10.3389/fendo.2013.00133>
- Messina A, Ferraris N, Wray S et al (2011) Dysregulation of Semaphorin7A/ $\beta$ 1-integrin signaling leads to defective GnRH-1 cell migration, abnormal gonadal development and altered fertility. *Hum Mol Genet* 20:4759–4774. <https://doi.org/10.1093/hmg/ddr403>
- Messina A, Pulli K, Santini S et al (2020) Neuron-derived neurotrophic factor is mutated in congenital hypogonadotropic hypogonadism. *Am J Hum Genet* 106:58–70. <https://doi.org/10.1016/j.ajhg.2019.12.003>
- Ming AYK, Yoo E, Vorontsov EN et al (2012) Dynamics and distribution of Klotho $\beta$  (KLB) and fibroblast growth factor receptor-1 (FGFR1) in living cells reveal the fibroblast growth factor-21 (FGF21)-induced receptor complex. *J Biol Chem*. <https://doi.org/10.1074/jbc.M111.325670>
- Miraoui H, Dwyer AA, Sykiotis GP et al (2013) Mutations in FGF17, IL17RD, DUSP6, SPRY4, and FLRT3 are identified in individuals with congenital hypogonadotropic hypogonadism. *Am J Hum Genet* 92:725–743. <https://doi.org/10.1016/j.ajhg.2013.04.008>
- Miura K, Acierno JS, Seminara SB (2004) Characterization of the human nasal embryonic LHRH factor gene, NELF, and a mutation screening among 65 patients with idiopathic hypogonadotropic hypogonadism (IHH). *J Hum Genet* 49:265–268. <https://doi.org/10.1007/s10038-004-0137-4>
- Monnier C, Dodé C, Fabre L et al (2009) PROKR2 missense mutations associated with Kallmann syndrome impair receptor signalling activity. *Hum Mol Genet* 18:75–81. <https://doi.org/10.1093/hmg/ddn318>
- Muscattelli F, Strom TM, Walker AP et al (1994) Mutations in the DAX-1 gene give rise to both X-linked adrenal hypoplasia congenita and hypogonadotropic hypogonadism. *Nature*. <https://doi.org/10.1038/372672a0>
- Nachtigall LB, Boepple PA, Pralong FP, Crowley WF (1997) Adult-onset idiopathic hypogonadotropic hypogonadism—a treatable form of male infertility. *N Engl J Med* 336:410–415. <https://doi.org/10.1056/NEJM199702063360604>
- Nakamura Y, Matsumoto H, Zaha K et al (2018) TUBB3 E410K syndrome with osteoporosis and cough syncope in a patient previously diagnosed with atypical Moebius syndrome. *Brain Dev* 40:233–237. <https://doi.org/10.1016/j.braindev.2017.12.006>
- Navarro VM (2013) Interactions between kisspeptins and neurokinin B. *Adv Exp Med Biol* 784:325–347. [https://doi.org/10.1007/978-1-4614-6199-9\\_15](https://doi.org/10.1007/978-1-4614-6199-9_15)
- Navarro VM, Gottsch ML, Chavkin C et al (2009) Regulation of gonadotropin-releasing hormone secretion by kisspeptin/dynorphin/neurokinin B neurons in the arcuate nucleus of the mouse. *J Neurosci* 29:11859–11866. <https://doi.org/10.1523/JNEUROSCI.1569-09.2009>
- Newbern K, Natrajan N, Kim H-G et al (2013) Identification of HESX1 mutations in Kallmann syndrome. *Fertil Steril* 99:1831–1837. <https://doi.org/10.1016/j.fertnstert.2013.01.149>
- Ng KL (2005) Dependence of olfactory bulb neurogenesis on Prokineticin 2 signaling. *Science* 308:1923–1927. <https://doi.org/10.1126/science.1112103>
- Nickell MD, Breheny P, Stromberg AJ, McClintock TS (2012) Genomics of mature and immature olfactory sensory neurons. *J Comp Neurol* 520:2608–2629. <https://doi.org/10.1002/cne.23052>
- Nunziata A, Borck G, Funcke J-B et al (2017) Estimated prevalence of potentially damaging variants in the leptin gene. *Mol Cell Pediatr*. <https://doi.org/10.1186/s40348-017-0074-x>
- Oleari R, Caramello A, Campinoti S et al (2019a) PLXNA1 and PLXNA3 cooperate to pattern the nasal axons that guide gonadotropin-releasing hormone neurons. *Development*. <https://doi.org/10.1242/dev.176461>
- Oleari R, Lettieri A, Paganoni A et al (2019b) Semaphorin signaling in GnRH neurons: from development to disease. *Neuroendocrinology* 109:193–199. <https://doi.org/10.1159/000495916>
- Oliveira LM, Seminara SB, Beranova M et al (2001) The importance of autosomal genes in Kallmann syndrome: genotype-phenotype correlations and neuroendocrine characteristics. *J Clin Endocrinol Metab* 86:1532–1538. <https://doi.org/10.1210/jcem.86.4.7420>
- Olsen SK, Li JYH, Bromleigh C et al (2006) Structural basis by which alternative splicing modulates the organizer activity of FGF8 in the brain. *Genes Dev*. <https://doi.org/10.1101/gad.1365406>
- Olson LE, Tollkuhn J, Scafoglio C et al (2006) Homeodomain-mediated  $\beta$ -catenin-dependent switching events dictate cell-lineage determination. *Cell*. <https://doi.org/10.1016/j.cell.2006.02.046>
- Palmert MR, Boepple PA (2001) Commentary: variation in the timing of puberty: clinical spectrum and genetic investigation. *J Clin Endocrinol Metab* 86:2364–2368
- Pask AJ, Kanasaki H, Kaiser UB et al (2005) A novel mouse model of hypogonadotropic hypogonadism: *N*-ethyl-*N*-nitrosourea-induced gonadotropin-releasing hormone receptor gene mutation. *Mol Endocrinol* 19:972–981. <https://doi.org/10.1210/me.2004-0192>
- Pasterkamp RJ, De Winter F, Holtmaat AJ, Verhaagen J (1998) Evidence for a role of the chemorepellent semaphorin III and its receptor neuropilin-1 in the regeneration of primary olfactory axons. *J Neurosci* 18:9962–9976
- Pfaeffle RW, Savage JJ, Hunter CS et al (2007) Four novel mutations of the LHX3 gene cause combined pituitary hormone deficiencies with or without limited neck rotation. *J Clin Endocrinol Metab*. <https://doi.org/10.1210/jc.2006-2177>
- Pfaeffle RW, Hunter CS, Savage JJ et al (2008) Three novel missense mutations within the LHX4 gene are associated with variable pituitary hormone deficiencies. *J Clin Endocrinol Metab* 93:1062–1071. <https://doi.org/10.1210/jc.2007-1525>
- Pingault V, Ente D, Le Moal FD et al (2010) Review and update of mutations causing Waardenburg syndrome. *Hum Mutat* 31(4):391–406. <https://doi.org/10.1002/humu.21211>
- Pingault V, Bodereau V, Baral V et al (2013) Loss-of-function mutations in SOX10 cause Kallmann syndrome with deafness. *Am J Hum Genet* 92:707–724. <https://doi.org/10.1016/j.ajhg.2013.03.024>
- Pitteloud N, Boepple PA, DeCruz S et al (2001) The fertile eunuch variant of idiopathic hypogonadotropic hypogonadism: spontaneous reversal associated with a homozygous mutation in the gonadotropin-releasing hormone receptor<sup>1</sup>. *J Clin Endocrinol Metab* 86:2470–2475. <https://doi.org/10.1210/jcem.86.6.7542>
- Pitteloud N, Hayes FJ, Boepple PA et al (2002) The role of prior pubertal development, biochemical markers of testicular maturation, and genetics in elucidating the phenotypic heterogeneity of idiopathic hypogonadotropic hypogonadism. *J Clin Endocrinol Metab* 87:152–160. <https://doi.org/10.1210/jcem.87.1.8131>
- Pitteloud N, Acierno JS, Meysing A et al (2006) Mutations in fibroblast growth factor receptor 1 cause both Kallmann syndrome and normosmic idiopathic hypogonadotropic hypogonadism. *Proc Natl Acad Sci USA*. <https://doi.org/10.1073/pnas.0600962103>
- Pitteloud N, Quinton R, Pearce S et al (2007a) Digenic mutations account for variable phenotypes in idiopathic hypogonadotropic hypogonadism. *J Clin Invest* 117:457–463. <https://doi.org/10.1172/JCI29884>

- Pitteloud N, Zhang C, Pignatelli D et al (2007b) Loss-of-function mutation in the prokineticin 2 gene causes Kallmann syndrome and normosmic idiopathic hypogonadotropic hypogonadism. *Proc Natl Acad Sci USA* 104:17447–17452. <https://doi.org/10.1073/pnas.0707173104>
- Pitteloud N, Durrani S, Raivio T, Sykiotis GP (2010) Complex genetics in idiopathic hypogonadotropic hypogonadism. Kallmann syndrome and hypogonadotropic hypogonadism. KARGER, Basel, pp 142–153
- Pozza SBD, Hiedl S, Roeb J et al (2012) A recessive mutation resulting in a disabling amino acid substitution (T194R) in the LHX3 homeodomain causes combined pituitary hormone deficiency. *Horm Res Paediatr* 77(1):41–51. <https://doi.org/10.1159/000335929>
- Prosser HM, Bradley A, Caldwell MA (2007) Olfactory bulb hypoplasia in Prokr2 null mice stems from defective neuronal progenitor migration and differentiation. *Eur J Neurosci* 26:3339–3344. <https://doi.org/10.1111/j.1460-9568.2007.05958.x>
- Quennell JH, Mulligan AC, Tups A et al (2009) Leptin indirectly regulates gonadotropin-releasing hormone neuronal function. *Endocrinology*. <https://doi.org/10.1210/en.2008-1693>
- Quinton R, Cheow HK, Tymms DJ et al (1999) Kallmann's syndrome: is it always for life? *Clin Endocrinol (Oxf)* 50:481–485. <https://doi.org/10.1046/j.1365-2265.1999.00708.x>
- Quinton R, Duke VM, Robertson A et al (2001) Idiopathic gonadotrophin deficiency: genetic questions addressed through phenotypic characterization. *Clin Endocrinol (Oxf)* 55:163–174. <https://doi.org/10.1046/j.1365-2265.2001.01277.x>
- Raivio T, Falardeau J, Dwyer A et al (2007) Reversal of idiopathic hypogonadotropic hypogonadism. *N Engl J Med* 357:863–873. <https://doi.org/10.1056/NEJMoa066494>
- Raivio T, Avbelj M, McCabe MJ et al (2012) Genetic overlap in kallmann syndrome, combined pituitary hormone deficiency, and septo-optic dysplasia. *J Clin Endocrinol Metab* 97:E694–E699. <https://doi.org/10.1210/jc.2011-2938>
- Ribeiro RS, Vieira TC, Abucham J (2007) Reversible Kallmann syndrome: report of the first case with a KAL1 mutation and literature review. *Eur J Endocrinol* 156:285–290. <https://doi.org/10.1530/eje.1.02342>
- Richards S, Aziz N, Bale S et al (2015) Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med* 17:405–424. <https://doi.org/10.1038/gim.2015.30>
- Robinson M, Parsons Perez MC, Tébar L et al (2004) FLRT3 is expressed in sensory neurons after peripheral nerve injury and regulates neurite outgrowth. *Mol Cell Neurosci*. <https://doi.org/10.1016/j.mcn.2004.06.008>
- Romero CJ, Pine-Twaddell E, Radovick S (2011) Novel mutations associated with combined pituitary hormone deficiency. *J Mol Endocrinol* 46(3):R93–R102. <https://doi.org/10.1530/JME-10-0133>
- Root AW (2010) Reversible isolated hypogonadotropic hypogonadism due to mutations in the neurokinin B regulation of gonadotropin-releasing hormone release. *J Clin Endocrinol Metab* 95:2625–2629. <https://doi.org/10.1210/jc.2010-0733>
- Ross RA, Leon S, Madara JC et al (2018) PACAP neurons in the ventral preamillary nucleus regulate reproductive function in the female mouse. *Elife*. <https://doi.org/10.7554/eLife.35960>
- de Roux N (2006) GnRH receptor and GPR54 inactivation in isolated gonadotropic deficiency. *Best Pract Res Clin Endocrinol Metab* 20:515–528. <https://doi.org/10.1016/j.beem.2006.10.005>
- de Roux N, Young J, Misrahi M et al (1997) A family with hypogonadotropic hypogonadism and mutations in the gonadotropin-releasing hormone receptor. *N Engl J Med* 337:1597–1603. <https://doi.org/10.1056/NEJM199711273372205>
- de Roux N, Young J, Brailly-Tabard S et al (1999) The same molecular defects of the gonadotropin-releasing hormone receptor determine a variable degree of hypogonadism in affected kindred<sup>1</sup>. *J Clin Endocrinol Metab* 84:567–572. <https://doi.org/10.1210/jcem.84.2.5449>
- de Roux N, Genin E, Carel J-C et al (2003) Hypogonadotropic hypogonadism due to loss of function of the KiSS1-derived peptide receptor GPR54. *Proc Natl Acad Sci* 100:10972–10976. <https://doi.org/10.1073/pnas.1834399100>
- de Tassigny XA, Fagg LA, Dixon JPC et al (2007) Hypogonadotropic hypogonadism in mice lacking a functional Kiss1 gene. *Proc Natl Acad Sci* 104:10714–10719. <https://doi.org/10.1073/pnas.0704114104>
- Rugarli EI, Ghezzi C, Valsecchi V, Ballabio A (1996) The Kallmann syndrome gene product expressed in COS cells is cleaved on the cell surface to yield a diffusible component. *Hum Mol Genet* 5:1109–1115. <https://doi.org/10.1093/hmg/5.8.1109>
- Salenave S, Chanson P, Bry H et al (2008) Kallmann's syndrome: a comparison of the reproductive phenotypes in men carrying KAL1 and FGFR1/KAL2 mutations. *J Clin Endocrinol Metab* 93:758–763. <https://doi.org/10.1210/jc.2007-1168>
- Santhakumar A, Balasubramanian R, Miller M, Quinton R (2014) Reversal of isolated hypogonadotropic hypogonadism: long-term integrity of hypothalamo-pituitary-testicular axis in two men is dependent on intermittent androgen exposure. *Clin Endocrinol (Oxf)* 81:473–476
- Sarfati J, Dodé C, Young J (2010) Kallmann syndrome caused by mutations in the PROK2 and PROKR2 genes: pathophysiology and genotype-phenotype correlations. *Front Horm Res* 39:121–132. <https://doi.org/10.1159/000312698>
- Sasaki G, Ogata T, Ishii T et al (2002) Novel mutation of TBX3 in a Japanese family with ulnar-mammary syndrome: implication for impaired sex development. *Am J Med Genet* 110:365–369. <https://doi.org/10.1002/ajmg.10447>
- Sato N, Katsumata N, Kagami M et al (2004) Clinical assessment and mutation analysis of Kallmann syndrome 1 (KAL1) and fibroblast growth factor receptor 1 (FGFR1, or KAL2) in five families and 18 sporadic patients. *J Clin Endocrinol Metab* 89:1079–1088. <https://doi.org/10.1210/jc.2003-030476>
- Sbai O, Monnier C, Dodé C et al (2014) Biased signaling through G-protein-coupled PROKR2 receptors harboring missense mutations. *FASEB J* 28:3734–3744. <https://doi.org/10.1096/fj.13-243402>
- Schang AL, Granger A, Quérat B et al (2013) GATA2-induced silencing and LIM-homeodomain protein-induced activation are mediated by a Bi-functional response element in the rat GnRH receptor gene. *Mol Endocrinol*. <https://doi.org/10.1210/me.2012-1182>
- Schilter KF, Schneider A, Bardakjian T et al (2011) OTX2 microphthalmia syndrome: four novel mutations and delineation of a phenotype. *Clin Genet*. <https://doi.org/10.1111/j.1399-0004.2010.01450.x>
- Schwanzel-Fukuda M, Bick D, Pfaff DW (1989) Luteinizing hormone-releasing hormone (LHRH)-expressing cells do not migrate normally in an inherited hypogonadal (Kallmann) syndrome. *Mol Brain Res* 6:311–326. [https://doi.org/10.1016/0169-328X\(89\)90076-4](https://doi.org/10.1016/0169-328X(89)90076-4)
- Schwartz GA, Raitcheva D, Bless EP et al (2004) Netrin 1-mediated chemoattraction regulates the migratory pathway of LHRH neurons. *Eur J Neurosci* 19:11–20. <https://doi.org/10.1111/j.1460-9568.2004.03094.x>
- Seeburg PH, Adelman JP (1984) Characterization of cDNA for precursor of human luteinizing hormone releasing hormone. *Nature* 311:666–668. <https://doi.org/10.1038/311666a0>
- Seifi M, Walter MA (2018) Axenfeld-Rieger syndrome. *Clin Genet* 93:1123–1130. <https://doi.org/10.1111/cge.13148>



- Seminara SB, Acierno JS, Abdulwahid NA et al (2002) Hypogonadotropic hypogonadism and cerebellar ataxia: detailed phenotypic characterization of a large, extended kindred. *J Clin Endocrinol Metab* 87:1607–1612. <https://doi.org/10.1210/jcem.87.4.8384>
- Seminara SB, Messager S, Chatzidaki EE et al (2003) The *GPR54* gene as a regulator of puberty. *N Engl J Med* 349:1614–1627. <https://doi.org/10.1056/NEJMoa035322>
- Sample RK, Achermann JC, Ellery J et al (2005) Two novel missense mutations in G protein-coupled receptor 54 in a patient with hypogonadotropic hypogonadism. *J Clin Endocrinol Metab* 90:1849–1855. <https://doi.org/10.1210/jc.2004-1418>
- Shaw ND, Brand H, Kupchinsky ZA et al (2017) SMCHD1 mutations associated with a rare muscular dystrophy can also cause isolated arhinia and Bosma arhinia microphthalmia syndrome. *Nat Genet* 49:238–248. <https://doi.org/10.1038/ng.3743>
- Sheng HZ, Moriyama K, Yamashita T et al (1997) Multistep control of pituitary organogenesis. *Science* 278:1809–1812. <https://doi.org/10.1126/science.278.5344.1809>
- Shi C-H, Schisler JC, Rubel CE et al (2014) Ataxia and hypogonadism caused by the loss of ubiquitin ligase activity of the U box protein CHIP. *Hum Mol Genet* 23:1013–1024. <https://doi.org/10.1093/hmg/ddt497>
- Sidhoum VF, Chan Y-M, Lippincott MF et al (2014) Reversal and relapse of hypogonadotropic hypogonadism: resilience and fragility of the reproductive neuroendocrine system. *J Clin Endocrinol Metab* 99:861–870. <https://doi.org/10.1210/jc.2013-2809>
- Simonis N, Migotte I, Lambert N et al (2013) FGFR1 mutations cause hartsfield syndrome, the unique association of holoprosencephaly and ectrodactyly. *J Med Genet*. <https://doi.org/10.1136/jmedgenet-2013-101603>
- Sinisi AA, Asci R, Bellastella G et al (2008) Homozygous mutation in the prokineticin-receptor2 gene (Val274Asp) presenting as reversible Kallmann syndrome and persistent oligozoospermia: case report. *Hum Reprod* 23:2380–2384. <https://doi.org/10.1093/humrep/den247>
- Sisodiya SM, Ragge NK, Cavalleri GL et al (2006) Role of SOX2 mutations in human hippocampal malformations and epilepsy. *Epilepsia* 47:534–542. <https://doi.org/10.1111/j.1528-1167.2006.00464.x>
- Smith JT, Acohido BV, Clifton DK, Steiner RA (2006) KiSS-1 neurones are direct targets for leptin in the ob/ob mouse. *J Neuroendocrinol* 18:298–303. <https://doi.org/10.1111/j.1365-2826.2006.01417.x>
- Soussi-Yanicostas N, Hardelin JP, Arroyo-Jimenez MM et al (1996) Initial characterization of anosmin-1, a putative extracellular matrix protein synthesized by definite neuronal cell populations in the central nervous system. *J Cell Sci* 109(Pt 7):1749–1757
- Soussi-Yanicostas N, de Castro F, Julliard AK et al (2002) Anosmin-1, defective in the X-linked form of Kallmann syndrome, promotes axonal branch formation from olfactory bulb output neurons. *Cell* 109:217–228. [https://doi.org/10.1016/s0092-8674\(02\)00713-4](https://doi.org/10.1016/s0092-8674(02)00713-4)
- Spilker C, Grochowska KM, Kreutz MR (2016) What do we learn from the murine Jacob/Nsmf gene knockout for human disease? *Rare Dis (Austin, Tex)* 4:e1241361. <https://doi.org/10.1080/21675511.2016.1241361>
- Srouf M, Riviere JB, Pham JMT et al (2010) Mutations in DCC cause congenital mirror movements. *Science* 328:592–592. <https://doi.org/10.1126/science.1186463>
- Stamou MI, Georgopoulos NA (2018) Kallmann syndrome: phenotype and genotype of hypogonadotropic hypogonadism. *Metabolism* 86:124–134. <https://doi.org/10.1016/j.metabol.2017.10.012>
- Stamou MI, Varnavas P, Plummer L et al (2019) Next-generation sequencing refines the genetic architecture of Greek GnRH-deficient patients. *Endocr Connect* 8:468–480. <https://doi.org/10.1530/EC-19-0010>
- Steevens AR, Sookiasian DL, Glatzer JC, Kiernan AE (2017) SOX2 is required for inner ear neurogenesis. *Sci Rep* 7:4086. <https://doi.org/10.1038/s41598-017-04315-2>
- Stenson PD, Mort M, Ball EV et al (2014) The human gene mutation database: building a comprehensive mutation repository for clinical and molecular genetics, diagnostic testing and personalized genomic medicine. *Hum Genet* 133:1–9
- Suh H, Gage PJ, Drouin J, Camper SA (2002) Pitx2 is required at multiple stages of pituitary organogenesis: pituitary primordium formation and cell specification. *Development* 129(2):329–337
- Swee DS, Quinton R (2019a) Congenital hypogonadotropic hypogonadism: minipuberty and the case for neonatal diagnosis. *Front Endocrinol (Lausanne)* 10:97. <https://doi.org/10.3389/fendo.2019.00097>
- Swee DS, Quinton R (2019b) Managing congenital hypogonadotropic hypogonadism: a contemporary approach directed at optimizing fertility and long-term outcomes in males. *Ther Adv Endocrinol Metab*. <https://doi.org/10.1177/2042018819826889>
- Sykiotis GP, Plummer L, Hughes VA et al (2010) Oligogenic basis of isolated gonadotropin-releasing hormone deficiency. *Proc Natl Acad Sci* 107:15140–15144. <https://doi.org/10.1073/pnas.1009622107>
- Synofzik M, Gonzalez MA, Lourenco CM et al (2014) PNPLA6 mutations cause Boucher-Neuhauser and Gordon Holmes syndromes as part of a broad neurodegenerative spectrum. *Brain* 137:69–77. <https://doi.org/10.1093/brain/awt326>
- Tajima T, Ishizu K, Nakamura A (2013) Molecular and clinical findings in patients with LHX4 and OTX2 mutations. *Clin Pediatr Endocrinol* 22(2):15–23. <https://doi.org/10.1292/cpe.22.15>
- Takagi M, Narumi S, Hamada R et al (2014) A novel KAL1 mutation is associated with combined pituitary hormone deficiency. *Hum Genome Var* 1:14011. <https://doi.org/10.1038/hgv.2014.11>
- Taniguchi K, Ayada T, Ichiyama K et al (2007) Sprouty2 and Sprouty4 are essential for embryonic morphogenesis and regulation of FGF signaling. *Biochem Biophys Res Commun*. <https://doi.org/10.1016/j.bbrc.2006.11.107>
- Tata B, Huijbregts L, Jacquier S et al (2014) Haploinsufficiency of Dmx12, encoding a synaptic protein, causes infertility associated with a loss of GnRH neurons in mouse. *PLoS Biol* 12:e1001952. <https://doi.org/10.1371/journal.pbio.1001952>
- Teles MG, Bianco SDC, Brito VN et al (2008) A GPR54-activating mutation in a patient with central precocious puberty. *N Engl J Med* 358:709–715. <https://doi.org/10.1056/NEJMoa073443>
- Tenenbaum-Rakover Y, Commenges-Ducos M, Iovane A et al (2007) Neuroendocrine phenotype analysis in five patients with isolated hypogonadotropic hypogonadism due to a L102P inactivating mutation of GPR54. *J Clin Endocrinol Metab* 92:1137–1144. <https://doi.org/10.1210/jc.2006-2147>
- Tétreault M, Choquet K, Orcesi S et al (2011) Recessive mutations in POLR3B, encoding the second largest subunit of Pol III, cause a rare hypomyelinating leukodystrophy. *Am J Hum Genet* 89:652–655. <https://doi.org/10.1016/j.ajhg.2011.10.006>
- Tiong J, Locastro T, Wray S (2007) Gonadotropin-releasing hormone-1 (GnRH-1) is involved in tooth maturation and biomineralization. *Dev Dyn* 236:2980–2992. <https://doi.org/10.1002/dvdy.21332>
- Tommiska J, Käsäkoski J, Christiansen P et al (2014) Genetics of congenital hypogonadotropic hypogonadism in Denmark. *Eur J Med Genet* 57:345–348. <https://doi.org/10.1016/j.ejmg.2014.04.002>
- Topaloglu AK, Reimann F, Guclu M et al (2009) TAC3 and TACR3 mutations in familial hypogonadotropic hypogonadism reveal a key role for Neurokinin B in the central control of reproduction. *Nat Genet* 41:354–358. <https://doi.org/10.1038/ng.306>
- Topaloglu AK, Tello JA, Kotan LD et al (2012) Inactivating KISS1 mutation and hypogonadotropic hypogonadism. *N Engl J Med* 366:629–635. <https://doi.org/10.1056/NEJMoa1111884>

- Topaloglu AK, Lomniczi A, Kretschmar D et al (2014) Loss-of-function mutations in PNPLA6 encoding neuropathy target esterase underlie pubertal failure and neurological deficits in Gordon Holmes syndrome. *J Clin Endocrinol Metab* 99:E2067–E2075. <https://doi.org/10.1210/jc.2014-1836>
- Tornberg J, Sykiotis GP, Keefe K et al (2011) Heparan sulfate 6-O-sulfotransferase 1, a gene involved in extracellular sugar modifications, is mutated in patients with idiopathic hypogonadotropic hypogonadism. *Proc Natl Acad Sci USA* 108:11524–11529. <https://doi.org/10.1073/pnas.1102284108>
- Trarbach EB, Silveira LG, Latronico AC (2007) Genetic insights into human isolated gonadotropin deficiency. *Pituitary* 10:381–391. <https://doi.org/10.1007/s11102-007-0061-7>
- Tsuchida T, Ensini M, Morton SB et al (1994) Topographic organization of embryonic motor neurons defined by expression of LIM homeobox genes. *Cell*. [https://doi.org/10.1016/0092-8674\(94\)90027-2](https://doi.org/10.1016/0092-8674(94)90027-2)
- Turan I, Hutchins BI, Hacıhamdioglu B et al (2017) CCDC141 mutations in idiopathic hypogonadotropic hypogonadism. *J Clin Endocrinol Metab* 102:1816–1825. <https://doi.org/10.1210/jc.2016-3391>
- Turton JPG, Mehta A, Raza J et al (2005) Mutations within the transcription factor PROP1 are rare in a cohort of patients with sporadic combined pituitary hormone deficiency (CPHD). *Clin Endocrinol (Oxf)*. <https://doi.org/10.1111/j.1365-2265.2005.02291.x>
- Vaaralahti K, Tommiska J, Tillmann V et al (2014) De novo SOX10 nonsense mutation in a patient with Kallmann syndrome and hearing loss. *Pediatr Res* 76:115–116. <https://doi.org/10.1038/pr.2014.60>
- Valdes-Socin H, Salvi R, Thiry A et al (2009) Testicular effects of isolated luteinizing hormone deficiency and reversal by long-term human chorionic gonadotropin treatment. *J Clin Endocrinol Metab*. <https://doi.org/10.1210/jc.2008-1584>
- Vezzoli V, Duminuco P, Bassi I et al (2016) The complex genetic basis of congenital hypogonadotropic hypogonadism. *Minerva Endocrinol* 41:223–239
- Villanueva C, Jacobson-Dickman E, Xu C et al (2015) Congenital hypogonadotropic hypogonadism with split hand/foot malformation: a clinical entity with a high frequency of FGFR1 mutations. *Genet Med*. <https://doi.org/10.1038/gim.2014.166>
- Wakabayashi Y, Nakada T, Murata K et al (2010) Neurokinin B and dynorphin A in kisspeptin neurons of the arcuate nucleus participate in generation of periodic oscillation of neural activity driving pulsatile gonadotropin-releasing hormone secretion in the goat. *J Neurosci* 30:3124–3132. <https://doi.org/10.1523/JNEUROSCI.5848-09.2010>
- Wang Y, Gong C, Qin M et al (2017) Clinical and genetic features of 64 young male paediatric patients with congenital hypogonadotropic hypogonadism. *Clin Endocrinol (Oxf)* 87:757–766. <https://doi.org/10.1111/cen.13451>
- Wang F, Zhao S, Xie Y et al (2018) De novo SOX10 nonsense mutation in a patient with Kallmann syndrome, deafness, iris hypopigmentation, and hyperthyroidism. *Ann Clin Lab Sci* 48:248–252
- Watanabe Y, Inoue K, Okuyama-Yamamoto A et al (2009) *Fezf1* is required for penetration of the basal lamina by olfactory axons to promote olfactory development. *J Comp Neurol* 515:565–584. <https://doi.org/10.1002/cne.22074>
- Weiss J, Adams E, Whitcomb RW et al (1991) Normal sequence of the gonadotropin-releasing hormone gene in patients with idiopathic hypogonadotropic hypogonadism. *Biol Reprod* 45:743–747. <https://doi.org/10.1095/biolreprod45.5.743>
- Welt CK, Chan JL, Bullen J et al (2004) Recombinant human leptin in women with hypothalamic amenorrhea. *N Engl J Med* 351:987–997. <https://doi.org/10.1056/NEJMoa040388>
- Whitman MC, Andrews C, Chan W-M et al (2016) Two unique TUBB3 mutations cause both CFEOM3 and malformations of cortical development. *Am J Med Genet A* 170A:297–305. <https://doi.org/10.1002/ajmg.a.37362>
- Williamson KA, Hever AM, Rainger J et al (2006) Mutations in SOX2 cause anophthalmia-esophageal-genital (AEG) syndrome. *Hum Mol Genet* 15:1413–1422. <https://doi.org/10.1093/hmg/ddl064>
- Wray S (2010) From nose to brain: development of gonadotrophin-releasing hormone -1 neurones. *J Neuroendocrinol* 22(7):743–753. <https://doi.org/10.1111/j.1365-2826.2010.02034.x>
- Wu S, Wilson MD, Busby ER et al (2010) Disruption of the single copy gonadotropin-releasing hormone receptor in mice by gene trap: severe reduction of reproductive organs and functions in developing and adult mice. *Endocrinology* 151:1142–1152. <https://doi.org/10.1210/en.2009-0598>
- Wyatt A, Bakrania P, Bunyan DJ et al (2008) Novel heterozygous OTX2 mutations and whole gene deletions in anophthalmia, microphthalmia and coloboma. *Hum Mutat*. <https://doi.org/10.1002/humu.20869>
- Xu N, Bhagavath B, Kim H-G et al (2010) NELF is a nuclear protein involved in hypothalamic GnRH neuronal migration. *Mol Cell Endocrinol* 319:47–55. <https://doi.org/10.1016/j.mce.2009.11.016>
- Xu N, Kim H-G, Bhagavath B et al (2011) Nasal embryonic LHRH factor (NELF) mutations in patients with normosmic hypogonadotropic hypogonadism and Kallmann syndrome. *Fertil Steril* 95(1613–20):e1–7. <https://doi.org/10.1016/j.fertnstert.2011.01.010>
- Xu C, Messina A, Somm E et al (2017) KLB, encoding  $\beta$ -Klotho, is mutated in patients with congenital hypogonadotropic hypogonadism. *EMBO Mol Med* 9:1379–1397. <https://doi.org/10.15252/emmm.201607376>
- Young J, Bouligand J, Francou B et al (2010) *TAC3* and *TACR3* defects cause hypothalamic congenital hypogonadotropic hypogonadism in humans. *J Clin Endocrinol Metab* 95:2287–2295. <https://doi.org/10.1210/jc.2009-2600>
- Young J, Metay C, Bouligand J et al (2012) SEMA3A deletion in a family with Kallmann syndrome validates the role of semaphorin 3A in human puberty and olfactory system development. *Hum Reprod* 27:1460–1465. <https://doi.org/10.1093/humrep/des022>
- Young J, Xu C, Papadakis GE et al (2019) Clinical management of congenital hypogonadotropic hypogonadism. *Endocr Rev* 40:669–710. <https://doi.org/10.1210/er.2018-00116>
- Zhang S, Cui W (2014) Sox2, a key factor in the regulation of pluripotency and neural differentiation. *World J Stem Cells* 6:305–311. <https://doi.org/10.4252/wjsc.v6.i3.305>
- Zhang Y, Proenca R, Maffei M et al (1994) Positional cloning of the mouse obese gene and its human homologue. *Nature*. <https://doi.org/10.1038/372425a0>
- Zhu J, Choa RE-Y, Guo MH et al (2015) A shared genetic basis for self-limited delayed puberty and idiopathic hypogonadotropic hypogonadism. *J Clin Endocrinol Metab* 100:E646–E654. <https://doi.org/10.1210/jc.2015-1080>

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