Genetics of Hypogonadotropic Hypogonadism—Human and Mouse Genes, Inheritance, Oligogenicity, and Genetic Counseling

Erica D. Louden, MD/PhD[a#]
Alexandra Poch, MD[a]
Hyung-Goo Kim, PhD[b]
Afif Ben-Mahmoud, PhD[b]
Soo-Hyun Kim, PhD[c]
Lawrence C. Layman, MD[a]

[a]Section of Reproductive Endocrinology, Infertility, & Genetics, Department of Obstetrics & Gynecology; Department of Neuroscience & Regenerative Medicine; Department of Physiology
Medical College of Georgia at Augusta University, Augusta, GA 30912
[b]Neurological Disorders Research Center, Qatar Biomedical Research Institute, Hamad Bin Khalifa University, Doha, Qatar.
[c]Molecular and Clinical Sciences Research Institute, St. George's Medical School, University of London Cranmer Terrace, London SW17 0RE, United Kingdom

#Present Address: VIOS Fertility, Chicago, IL

Please address correspondence to E.D. Louden or L.C. Layman at:
Section of Reproductive Endocrinology, Infertility, & Genetics, Department of Obstetrics & Gynecology
Medical College of Georgia at Augusta University, 1120 15th Street, Augusta, GA 30912, (706)721-3832

Conflicts of interest: The authors have no conflicts of interest

Acknowledgements: NIH HD33004 to LCL from 1997-2017, multiple collaborators, and particularly Lynn Chorich, M.S., Lab Manager in the Layman Lab; portions of this were presented at the Endocrine Society, 2018

This manuscript is dedicated to Paul G. McDonough, M.D., mentor and pioneer in the genetics of reproductive disorders, who understood long ago that molecular genetics was the key to understanding reproductive processes.

Abstract

Hypogonadotropic hypogonadism, which may be normosmic (nHH) or anosmic/hyposmic, known as Kallmann syndrome (KS), is due to gonadotropin-releasing hormone deficiency, which results in absent puberty and infertility. Investigation of the genetic basis of nHH/KS over the past 35 years has yielded a substantial increase in our understanding, as variants in 44 genes in OMIM account for ~50% of cases. The first genes for KS (*ANOS1*) and nHH (*GNRHR*) were followed by the discovery that *FGFR1* variants may cause either nHH or KS. Associated anomalies include midline facial defects, neurologic deficits, cardiac anomalies, and renal agenesis, among others. Mouse models for all but one gene (*ANOS1*) generally support findings in humans. About half of the known genes implicated in nHH/KS are inherited as autosomal dominant and half are autosomal recessive, whereas only 7% are X-linked recessive. Digenic and oligogenic inheritance has been reported in 2-20% of patients, most commonly with variants in genes that may result in either nHH or KS inherited in an autosomal dominant fashion. *In vitro* analyses have only been conducted for both gene variants in eight cases and for one gene variant in 20 cases. Rigorous confirmation that two gene variants in the same individual cause the nHH/KS phenotype is lacking for most. Clinical diagnosis is probably best accomplished by targeted next generation sequencing of the known candidate genes with confirmation by Sanger sequencing. Elucidation of the genetic basis of nHH/KS has resulted in an enhanced understanding of this disorder, as well as normal puberty, which makes genetic diagnosis clinically relevant.

1.0 Introduction

This review will focus on an overview of the genetics of hypogonadotropic hypogonadism, including both normosmic hypogonadotropic hypogonadism (nHH) and Kallmann syndrome (KS). We will discuss the known causative genes succinctly and provide concepts and categories of genes along with genotype/phenotype correlations. We will only include those genes recognized by Online Mendelian Inheritance in Man (OMIM) as causative, but 14 newer genes of uncertain significance (GUS) will also be mentioned. As per the American College of Medical Genetics and Genomics, the word "mutations" will be replaced by "variants." Variants are classified as "pathogenic," "likely pathogenic," "uncertain significance," "likely benign," and "benign" (Richards et al. 2015). Pathogenic variants are defined as those variants in a known gene that segregate with the phenotype (present in affected patients and absent in unaffected patients) and are supported by *in vitro* studies. They are typically nonsense, frameshift, and splice site, but may be missense if proven *in vitro* in a known causative role. It should be noted that these classifications correspond to those genes already known to be associated with the phenotype of nHH/KS, i.e., those with MIM numbers. For GUS variants that do not yet have MIM numbers, variants will be considered as "variants of uncertain significance (VUS) (Richards et al. 2015).

Relevant mouse models are shown for comparison, including the phenotypes of both heterozygotes and homozygotes. Associated phenotypic findings along with Mendelian inheritance patterns will be shown. Reported digenic variants will also be critically evaluated, including evidence from human, mouse, and cellular studies. Lastly, practical genetic testing of patients will be discussed. This review will focus on the newest genetic aspects of nHH/KS, which will update, integrate, and expand upon recent reviews (Boehm et al. 2015; Cho et al. 2019; Maione et al. 2018). We believe this to be the first comprehensive analysis of putative digenic disease in nHH/KS.

1.1 Development and Function of the Hypothalamic-Pituitary-Gonadal Axis

Embryonic development of gonadotropin-releasing hormone (GnRH)-secreting and olfactory neurons are interdependent and initially occur outside of the brain, a process conserved across vertebrates. After their fate specification from the pre-placodal ectoderm, GnRH neurons migrate out of the developing vomeronasal organ along the olfactory axonal projections and olfactory ensheathing cells, forming a bundle, until they reach the cribriform plate (Cho et al. 2019). This bundle then converges with axons and olfactory ensheathing cells from the main olfactory epithelium. Axons from olfactory neurons travel rostrally to the olfactory bulb, while GnRH neurons turn ventrally along a transient neuronal pathway (the terminal nerve) to reach the hypothalamus (Cho et al. 2019). Cell bodies for GnRH neurons affecting reproduction are principally located in the arcuate nucleus of the hypothalamus and they send projections to the median eminence where GnRH enters the hypophyseal-portal system (Cho et al. 2019).

The normal reproductive axis depends upon pulsatile GnRH from the hypothalamus, which is secreted into the hypophyseal-portal system and binds to its membrane bound receptor on the surface of pituitary gonadotropes. The pituitary responds with the synthesis and secretion of gonadotropins follicle stimulating hormone (FSH) and luteinizing hormone (LH). Both FSH and LH bind their cell surface receptors in the gonads to stimulate the production of sex steroids (estradiol in females; testosterone in males) and gametes (oocytes and spermatozoa). The sex steroids exert negative feedback at both the hypothalamus and pituitary (Layman 1999a). Kisspeptin is a key inducer of the pubertal surge of sex hormones as it stimulates GnRH release upon binding to kisspeptin receptors present on GnRH neurons (Seminara 2007; Messager et al. 2005). Other higher brain centers also regulate GnRH secretion, and a number of growth factors are also known to participate in HPG axis function (Divall et al. 2010).

1.2 The Phenotype of Hypogonadotropic Hypogonadism

Hypogonadotropic hypogonadism is defined as low serum levels of gonadotropins and sex steroids in individuals with absent/impaired puberty at age 17-18 in females and 18 in males (Crowley et al. 1985; Layman 2002). In nHH/KS, sex steroids are low (or in the low normal range), and with a lack of negative feedback to the hypothalamus and pituitary, the measurement of serum FSH and LH helps localize the defect within the HPG axis. Since FSH and LH are low (or inappropriately normal) in nHH/KS, a hypothalamic (more commonly) or pituitary defect is present. Further support for a hypothalamic etiology in nHH/KS comes from studies of impaired LH pulsatility as a consequence of GnRH deficiency (Santoro, Filicori, and Crowley 1986).

There are a number of terms for hypogonadotropic hypogonadism in the literature—GnRH deficiency, isolated gonadotropin deficiency, and more recently idiopathic hypogonadotropic hypogonadism (IHH) and congenital hypogonadotropic hypogonadism. Since the etiology is now known for about half of cases, we will replace IHH with either normosmic hypogonadotropic hypogonadism (nHH) or Kallmann syndrome (KS). Kallmann syndrome is characterized by the combination of GnRH deficiency with anosmia/hyposmia due to impaired GnRH neuron migration. In addition to its characterization based upon sense of smell, nHH/KS may be categorized by the degree of pubertal development impairment—complete if no pubertal development or incomplete if there is partial pubertal development. It is also well recognized that adult onset forms of nHH/KS have been reported and that some patients have pathogenic variants in known nHH/KS genes (Boehm et al. 2015; Caronia et al. 2011; Santoro, Filicori, and Crowley 1986) due to impaired GnRH neuron migration or GnRH action.

In the literature, there is a male preponderance of patients diagnosed with nHH/KS, typically 3:1 to 5:1, and nHH/KS is usually (but not always) irreversible (Boehm et al. 2015). Females typically have absent breast development (or partial development) along with amenorrhea. Males lack the testosterone-induced voice change and usually have small testes, and some may manifest cryptorchidism. In the clinical evaluation of these patients with FSH and LH, hypopituitarism must be excluded, and it is now well known that pathogenic variants in some genes result in combined pituitary hormone deficiency (CPHD), a key phenotypic feature of which is short stature (Layman 2013b; Trofimova et al. 2017). It is important to remember that a number of somatic anomalies may accompany nHH/KS including midline facial defects, neurologic abnormalities such as synkinesia or ataxia, visual or hearing defects as well as cardiac defects and renal agenesis (Layman 1999a; Boehm et al. 2015). Since gonadal function is typically present, these patients respond to exogenous GnRH or gonadotropin therapy so pregnancy is quite possible. An understanding of the genetic types of nHH/KS is therefore of critical importance to counsel patients regarding the risk of transmission.

2.0 Human nHH/KS Genes

2.1Kallmann Syndrome—First Gene

Three important genes deserve particular attention since they constituted the first descriptions of X-linked, autosomal recessive, and autosomal dominant forms of nHH/KS. Information regarding the genetic basis of nHH/KS has exploded since the early 1990s with the initial discovery of the Kallmann syndrome-1 gene—first known as *KALIG1* and *ADMLX*, then *KAL1*, and now known as *ANOS1,* which encodes the protein anosmin-1 (Franco et al. 1991; Legouis et al. 1991). We would expect that a KS gene would encode a protein directing the migration of both GnRH and olfactory neurons, and a pathogenic variant would result in GnRH deficiency and anosmia.

Through deletion mapping of the short arm of the X chromosome, *ANOS1* deletions and point pathogenic variants were identified in males with KS (Franco et al. 1991; Legouis et al. 1991; Bick et al. 1992). The discovery of *ANOS1* was facilitated in males with KS who also had coexistent ichthyosis which is caused by steroid sulfatase (*STS*) gene deletions, the nearby gene that was already characterized. *ANOS1* pathogenic variants are inherited in an X-linked recessive fashion thereby affecting only males, which interestingly is the least common inheritance pattern of nHH or KS in humans. With detailed phenotyping, ~10% of males with *ANOS1* pathogenic variants were also found to possess unilateral renal agenesis, and ~25% demonstrate synkinesia (Quinton et al. 2001). Interestingly, no mouse ortholog for *ANOS1* has yet been identified, although found in chicks, zebrafish, and C. elegans. The phenotype of *ANOS1* pathogenic variants correlates very closely with the expression patterns in the chick, as shown in Table 1 (Rugarli and Ballabio 1993). In addition to olfactory neuron expression, expression in the CNS explains nystagmus, synkinesia, and eye movement abnormalities (Rugarli and Ballabio 1993; Layman 1999b). *ANOS1* is also expressed in the facial mesenchyme and in the kidney, corresponding to the somatic anomalies that may accompany KS. (Rugarli and Ballabio 1993; Layman 1999b) Pathogenic variants in *ANOS1* occur in up to 50% of clearly inherited X-linked recessive families (Hardelin et al. 1993), but only comprise about 5-10% of males in non-X-linked families (Layman 2013a).

2.2 Normosmic HH—First Gene

The first gene identified to possess pathogenic variants in patients with nHH was the gonadotropin releasing hormone receptor (*GNRHR*) gene. One group (de Roux et al. 1997) studied a family with incomplete pubertal development and identified compound heterozygous *GNRHR* pathogenic variants that impaired signaling *in vitro*. About the same time, another group (Layman et al. 1998) studied 46 nHH probands and found one family with four affected siblings (three women and one man) who completely lacked pubertal development. One female had no breast development at age 30 and failed to respond to increasing doses of GnRH, suggesting GnRH resistance. Her two sisters lacked breast development and her brother had no voice change, and had low serum testosterone. FSH and LH levels were low or normal. Each affected family member had compound heterozygous *GNRHR* pathogenic variants that impaired signaling *in vitro* (Layman et al. 1998). *GNRHR* pathogenic variants result in nHH, but the effect upon pubertal development could be variable, ranging from partial to complete impairment. Patients with *GNRHR* pathogenic variants have not been found to have anosmia or other associated anomalies, and in two large series, they comprise ~4-5% of the genetic etiology on nHH (Bhagavath et al. 2005; Francou et al. 2016). *GNRHR* was the first gene shown to have pathogenic variants that affect females and was the first autosomal locus for nHH, being inherited in an autosomal recessive fashion.

2.3 Both nHH/KS—First Gene

The first autosomal dominantly inherited form to result in either phenotype—nHH or KS—was due to pathogenic variants in the fibroblast growth factor receptor-1 (*FGFR1*) gene, as determined by positional cloning in patients with overlapping deletions (Dode et al. 2003) and positional cloning of a balanced translocation (Kim et al. 2005). It is remarkable that activating pathogenic variants of *FGFR1* were previously identified in craniosynostoses—Pfeiffer syndrome (MIM# 101600) and Jackson-Weiss syndrome (MIM# 123150), but also in some rare severe syndromes—Trigonocephaly (MIM# 190440), Osteoglophonic Dysplasia (MIM# 166250), and Hartsfield syndrome (MIM# 615465). Inactivating *FGFR1* pathogenic variants were found first in KS,(Dode and Hardelin 2004) but subsequently in nHH (Pitteloud, Zhang, et al. 2007), making it the first gene in which pathogenic variants caused either nHH or KS (Pitteloud, Acierno, et al. 2006). *FGFR1* pathogenic variants demonstrate reduced penetrance and variable expressivity, which are characteristic of autosomal dominant disorders. Unlike *GNRHR* pathogenic variants, patients with *FGFR1* pathogenic variants may have other associated anomalies including dental agenesis, cleft lip/palate, and synkinesia (Dode et al. 2003; Pitteloud, Meysing, et al. 2006). Pathogenic variants in *FGFR1* are probably the most commonly encountered in nHH/KS and comprise ~10%. It should be noted that the phenotype of males with *FGFR1* variants is usually less severe compared with *ANOS1* variants. Males with *ANOS1* variants are more likely to have cryptorchidism, smaller testicular volume, and lower mean baseline and GnRH-stimulated LH levels (Salenave et al. 2008), while males with *FGRF1* variants manifest a broader phenotypic spectrum from normal puberty to delayed puberty to hypogonadotropic hypogonadism, reflecting variable expressivity (Pitteloud, Meysing, et al. 2006).

2.4 Additional nHH/KS Genes

Shown in Table 2 and Supplemental Figure 1 are the 44 genes recognized by OMIM known to be involved in the etiology in ~50% of nHH/KS with pathogenic variants confirmed by *in vitro* analysis. The chromosomal locus is shown in Figure 1. In addition, there are 14 GUS, which are likely to be included in OMIM in the future when more supportive evidence is provided. Seven genes were either recognized by the European Consensus Group (Boehm et al. 2015) (*OTUD4 (Margolin et al. 2013), SEMA7A* (Kansakoski et al. 2014), and *AXL (Salian-Mehta et al. 2014)*) or included in another recent review (Cho et al. 2019) (*CCDC141 (Hutchins et al. 2016), KLB (Xu et al. 2017), PLXNA1 (Kotan et al. 2019),* and *TUBB3(Chew et al. 2013)*). The last seven genes—*ROBO1 (Zhu et al. 2020), ROBO2 (Zhu et al. 2020), SCEL (Zhu et al. 2020), SRA1 (Kotan et al. 2016), TBX3 (Galazzi et al. 2018), IGSF10 (Howard et al. 2016),* and *PTCH1 (Barraud et al. 2021)* have been characterized with a phenotype of nHH/KS more recently, and will not be further discussed here.

Pathogenic variants in most genes affect the hypothalamus, while genes such as *NR0B1* affect both hypothalamic and pituitary function (Habiby et al. 1996). Still others, largely homeobox genes, cause combined pituitary hormone deficiency (CPHD), which includes gonadotropin deficiency (Layman 2013b).It stands to reason that pathogenic variants in a gene encoding a ligand and its receptor could lead to a similar phenotype.

For example, protein products of *ANOS1(Legouis et al. 1991; Franco et al. 1991)* and *FGF8(Falardeau et al. 2008)* both bind to the FGFR1 protein and utilize heparan sulfate (involving a sulfotransferase gene--*HS6ST1(Tornberg et al. 2011)*). Pathogenic variants in other FGF pathway genes have also been substantiated in patients in nHH/KS, including *FGF17, IL17RD, DUSP6, SPRY4,* and *FLRT3*(Miraoui et al. 2013), as well as *FGF8(Falardeau et al. 2008)* (Table 2). Recently, it has also been shown that anosmin-1 can interact with PROKR2, suggesting a link between these two common nHH/KS pathways (Murcia-Belmonte, Astillero-Lopez, and Esteban 2016).

The genes in the FGF/PROKR2 pathway appear to be involved in GnRH and olfactory neuron migration, and pathogenic variants often cause KS. In contrast, pathogenic variants in other ligand-receptor gene pairs such as the genes for GnRH (*GNRH1(Chan et al. 2009; Bouligand et al. 2009)*), leptin (*LEP(Strobel et al. 1998; Montague et al. 1997)*), kisspeptin (*KISS1(Topaloglu et al. 2012)*), and neurokinin B (*TAC3(Topaloglu et al. 2009)*), as well as their receptors (*GNRHR (de Roux et al. 1997; Layman et al. 1998), LEPR (Clement et al. 1998), KISS1R* (de Roux et al. 2003; Seminara et al. 2003), and *TACR3(Topaloglu et al. 2009)*) cause nHH, suggesting their principle effect is upon GnRH regulation/secretion. Kisspeptin, Neurokinin B, and Dynorphin are found in KNDy hypothalamic neurons which regulate GnRH secretion (Burke et al. 2006). Typically, pathogenic variants in the receptor genes are more common than those in the ligand. Initially, kisspeptin was known as metastin in the cancer literature, but largely unknown in reproductive endocrinology until the discovery of the ligand and its receptor as causative nHH genes (Seminara et al. 2003; de Roux et al. 2003). They are important because they perhaps best exemplify how finding gene pathogenic variants in rare disorders can profoundly impact our understanding of normal physiology. Kisspeptin neurons interconnect with GnRH neurons and are involved in both positive and negative feedback of estradiol upon gonadotropin secretion (Burke et al. 2006).

Pathogenic variants in some genes only cause KS such as *ANOS1* (Franco et al. 1991; Legouis et al. 1991), *SEMA3A(Young et al. 2012; Hanchate et al. 2012), SOX10 (Pingault et al. 2013), HESX1 (Dattani et al. 1998),* and *FEZF1(Kotan et al. 2014),* suggesting principal effects upon GnRH neuron specification/migration. MRI evidence of olfactory bulb absence or hypoplasia may be present with anosmia, and although more likely with *ANOS1* variants, *PROKR2* and *PROK2* VUS have been associated with absent olfactory bulbs (Moya-Plana et al. 2013). Overall, it is difficult at this time to make genotype/phenotype correlations with olfactory tract abnormalities.

Other genes regulate GnRH secretion/signaling and only result in nHH, such as *GNRH1 (Chan et al. 2009; Bouligand et al. 2009), GNRHR (de Roux et al. 1997; Layman et al. 1998), LEP (Strobel et al. 1998; Montague et al. 1997), LEPR (Clement et al. 1998), KISS1 (Topaloglu et al. 2012), KISS1R (de Roux et al. 2003; Seminara et al. 2003), TAC3*(Topaloglu et al. 2009), *TACR3*(Topaloglu et al. 2009), *PCSK1 (Jackson et al. 1997), DMXL2(Tata et al. 2014), RNF216 (Margolin et al. 2013), PNPLA6 (Topaloglu et al. 2014),* and *NR0B1(Muscatelli et al. 1994; Zanaria et al. 1994)* (see Table 2 for a complete list of genes). However, pathogenic variants in some genes, such as *FGF8 (Falardeau et al. 2008), FGFR1 (Pitteloud, Zhang, et al. 2007), FGF17 (Miraoui et al. 2013), IL17RD* (Miraoui et al. 2013), *PROK2 (Dode et al. 2006; Sarfati et al. 2010), PROKR2 (Dode et al. 2006; Sarfati et al. 2010), CHD7 (Kim et al. 2008), WDR11 (Kim et al. 2010), HS6ST1* (Tornberg et al. 2011), and *NSMF(Pitteloud, Quinton, et al. 2007; Xu et al. 2011)* may cause either KS or nHH suggesting they could have important functions in both GnRH neuron specification/migration and GnRH regulation/secretion (Table 2). Certainly as more cases are described in the literature, the breadth of the phenotypes will likely be expanded.

Pathogenic variants in nHH/KS may be associated with other nonreproductive anomalies as already mentioned for *ANOS1(Franco et al. 1991; Legouis et al. 1991)* and *FGFR1(Dode et al. 2003)* (Table 3). Variants in *LEP(Strobel et al. 1998; Montague et al. 1997)* and the *LEPR(Clement et al. 1998)* result in nHH, but also hyperphagia and morbid obesity; although only a small percentage of morbidly obese individuals have pathogenic variants in *LEP(Strobel et al. 1998; Montague et al. 1997)* or *LEPR(Clement et al. 1998)* genes. *NR0B1* pathogenic variants result in adrenal hypoplasia congenita in males (X-linked recessive), and treated males manifest nHH later (Zanaria et al. 1994; Muscatelli et al. 1994).

Another common nHH/KS gene—*CHD7*—was first known to be involved in the pathogenesis of CHARGE syndrome, which includes at least four of six Blake criteria: Coloboma of the eye, Heart malformations, Atresia of the choanae, Retardation of growth and development, Genital anomalies, and Ear abnormalities and deafness

(Vissers et al. 2004). Could nHH/KS be a mild allelic variant of CHARGE syndrome since olfactory dysfunction and hypogonadism may be constituents of both KS and CHARGE? This appears to be the case since ~6% of nHH/KS patients without full CHARGE criteria have heterozygous *CHD7* pathogenic variants, which is now known to be involved in the GnRH system (Kim et al. 2008), and *CHD7* along with *FGFR1* (Falardeau et al. 2008; Pitteloud, Acierno, et al. 2006), are the two of the most common genes to have pathogenic variants in nHH/KS (Layman 2013a; Amato et al. 2019; Cassatella et al. 2018). Pathogenic variants in *WDR11*, which was mapped by positional cloning utilizing a patient with a balanced translocation, interfere with EMX1 binding (Kim et al. 2010) and by virtue of its involvement in the sonic hedgehog pathway, support that the disorder is a ciliopathy (Kim et al. 2018). Interestingly, recently identified variants (VUS) in *PTCH1* also support a role for the hedgehog pathway (Barraud et al. 2021).

Pathogenic variants in at least four genes cause deafness (*SOX10* (Pingault et al. 2013), *CHD7 (Vissers et al. 2004; Kim et al. 2008), DMXL2,(Tata et al. 2014),* and *IL17RD(Miraoui et al. 2013)*), while pathogenic variants in two genes in the ubiquitin pathway—*RNF216*(Margolin et al. 2013) and *STUB1(Shi et al. 2014)*—result in nHH with ataxia in a syndrome known as Gordon-Holmes Syndrome (also *OTUD4*, a GUS, also seems to be involved (Margolin et al. 2013)). *PNPLA6* pathogenic variants also cause ataxia with nHH resulting in either Gordon-Holmes Syndrome or Boucher-Neuhauser Syndrome (Table 3) *(Topaloglu et al. 2014).* Several other genes deserve special mention. *SMCHD1* has been known to be involved in the etiology of some cases of fascioscapulohumeral muscular dystrophy type 2 (FSHD2), but more recently, pathogenic variants have been characterized in patients with a rare ophthalmologic disorder with accompanying nHH—Bosma Arhinia Microphthlamia Syndrome (Shaw et al. 2017). Pathogenic variants in *POLR3A* and *POLR3B* cause nHH with the 4H syndrome (hypomyelination, hypogonadotropic hypogonadism, and hypodontia) (Daoud et al. 2013).

There are at least six homeobox genes (*PROP1 (Wu et al. 1998), HESX1 (Dattani et al. 1998), SOX2 (Kelberman et al. 2006), SOX3 (Laumonnier et al. 2002), LHX3* (Netchine et al. 2000), and *LHX4*)(Machinis et al. 2001; Tajima et al. 2007) involved in combined pituitary hormone deficiency (CPHD), which include gonadotropin deficiency and short stature. Some have other associated anomalies such as *SOX2* (eye, hearing loss, septo-optic dysplasia) (Kelberman et al. 2006), *SOX3* (isolated growth hormone deficiency and intellectual disability) (Laumonnier et al. 2002), *LHX3* (short cervical spine, variable pituitary size) (Netchine et al. 2000), and *LHX4* (isolated growth hormone deficiency, abnormal cerebellum, small or ectopic pituitary) (Machinis et al. 2001; Tajima et al. 2007). Patients with pathogenic variants in *HESX1* may manifest KS with midline facial defects including septo-optic dysplasia (Dattani et al. 1998). Additionally, rare isolated deficiencies of the beta subunit genes encoding FSHβ(Matthews et al. 1993; Layman et al. 1997) and LHβ(Valdes-Socin et al. 2004; Lofrano-Porto et al. 2007) and have also been reported. In each case, the protein encoded by the defective subunit is low (low serum LH with *LHB* pathogenic variants (Valdes-Socin et al. 2004; Lofrano-Porto et al. 2007); low serum FSH with *FSHB* pathogenic variant (Matthews et al. 1993; Layman et al. 1997)), while the unaffected gonadotropin is elevated. This is because both dimeric gonadotropin proteins possess the same alpha subunit, but pathogenic variants in specific beta subunits impair intact dimer formation.

2.5 Hypothalamic and Pituitary Effects

As already mentioned, *NR0BIv(Habiby et al. 1996)* variants may impair hypothalamic, pituitary, and adrenal function, but this gene also has a gonadal actionv(Yu et al. 1998). Since there is broad expression of many genes, there is growing evidence suggesting that some nHH/KS genes could have gonadal effects. Rarely, pathogenic variants in each of the following genes have been shown to cause pituitary hypofunction in addition to their known role in the hypothalamus—*FGFR1 (Simonis et al. 2013), FGF8(McCabe et al. 2011), PROKR2 (McCormack et al. 2017), HESX1 (Dattani et al. 1998; Newbern et al. 2013),* and *WDR11* (Cole et al. 2008). Testing pituitary function in patients with nHH/KS is prudent, but particularly when variants are identified for these genes in patients, clinical evaluation should include testing for pituitary insufficiency, which can be life threatening if untreated.

2.6 Reversible nHH/KS

It has now become recognized that reversibility of nHH/KS may occur in some patients. In this case, affected individuals who responded to treatment with GnRH, gonadotropins, or sex steroids for their hypogonadism continue to manifest normal gonadal function once the therapy is discontinued. The first report

of reversibility of an nHH patient with a pathogenic variant in a known gene involved *GNRHR (Pitteloud et al. 2001; Lin et al. 2006)*, which was followed by reversibility of KS (Pitteloud et al. 2005) and nHH(Raivio et al. 2009) with *FGFR1* pathogenic variants. Other reported gene variants exhibiting reversible nHH/KS include: *ANOS1* (Ribeiro, Vieira, and Abucham 2007), *PROKR2* (Sinisi et al. 2008; Cole et al. 2008), *TAC*3 (Gianetti et al. 2010), *TACR3* (Gianetti et al. 2010), *CHD7* (Laitinen et al. 2012), and *SOX10* (Maione et al. 2016). In the largest study to date, 44/308 (22%) of nHH/KS patients demonstrated reversibility, and on occasion, relapse (Sidhoum et al. 2014). These included patients with *FGFR1, PROKR2,* and *GNRHR* pathogenic variants (no patient with *ANOS1* pathogenic variants). *TAC3* and *TACR3* pathogenic variants comprised about half of those that showed reversibility (Sidhoum et al. 2014). When a subset of seven patients with pathogenic biallelic *TACR3* variants was examined, three of seven demonstrated reversal, while three of seven with heterozygous variants demonstrated evidence for reversibility of their hypogonadism (Gianetti et al. 2010). Therefore, counseling of patients, particularly with pathogenic variant in these genes, should include the possibility for reversal and relapse.

3.0 Genome Wide Association Studies (GWAS) and Hypogonadotropic Hypogonadism

The nHH/KS genes discussed in this review involve the identification of specific variants in genes that interfere with function and underlie the pathogenesis of the disorder. In contrast, genome wide association studies (GWAS) have been used to dissect regions of the genome that are associated common traits such as age at menarche, age at first birth, age at menopause, or infertility. GWAS consists of a case-control association of multiple DNA markers scattered across the genome with the phenotype of interest. Since effect sizes for common variants are small, large sample sizes are needed. It should be realized that GWAS identifies genomic regions or regions near genes (not necessarily within the protein coding regions of the gene) associated with a particular trait rather than specific genes and pathways (Gajbhiye, Fung, and Montgomery 2018). The eventual goal of GWAS is to identify specific genes that impair function, but this has already been elucidated for more than half of patients with nHH/KS by different approaches.

Although some of the genetic regions associated with reproductive milestones such as age at menarche and age at menopause involve reproductive genes, including nHH/KS genes, others do not. For example, DNA damage/repair, immune response and breast cancer processes genes are associated with age at menopause, as are nHH/KS genes—*CHD7*, *FGFR1*, *SOX10*, and *TAC3* (Day et al. 2015). Associated loci explain about 25% of the heritability of the age of menarche (Day et al. 2017). Approximately 250 genes expressed in neural tissues were associated with age of menarche, with two genes for precocious puberty—*MKRN3* and *DLK1*—having a large effect if paternally expressed (it is known that paternal transmission of variants in these genes result in precocious puberty) (Abreu et al. 2013). Causal associations were suggested between puberty timing and the increased risk of cancer (breast and endometrial cancer in women and prostate cancer in men) (Day et al. 2017).

For one of the nHH/KS genes associated with nHH/KS, SNPs in other parts of the gene have shown association in non-nHH/KS situations. SNPs upstream of the transcription start site of *FSHB* (so are not located in the protein coding region as has been described for isolated FSH deficiency) are associated with increased levels of circulating FSH, decreased LH levels, shorter menstrual cycles, increased dizygotic twinning, increased risk of endometriosis, decreased risk of PCOS, and earlier menopause (Gajbhiye, Fung, and Montgomery 2018). GWAS has not been typically used for nHH/KS because of its more obvious Mendelian inheritance (despite digenicity), but has been useful for complex disorders such as PCOS, endometriosis, and diabetes.

4.0 Mouse Models

Mouse models now exist for 43 of the 44 known human nHH/KS genes (no *ANOS1* gene is found in mice) (Table 4, which also includes the references and PMID). *A priori* expectations would be that homozygous knockout mice manifest the phenotype of hypogonadotropic hypogonadism, while heterozygotes would be unaffected. However, this is very variable as shown in Figure 2 and Table 4. The first known model was the hypogonadal mouse, published more than 30 years ago, which had a naturally occurring deletion of much of the *Gnrh1* gene.(Mason, Hayflick, et al. 1986) Although protein sequences for the decapeptide were not deleted, the precursor transcript was unstable, and exogenous GnRH administration or GnRH gene therapy corrected the

defect (Mason, Pitts, et al. 1986). Others followed and the phenotypes generally agree with human phenotypes, but not always.

When all 43 mouse models are examined, 22/43 (51%) of the homozygotes were embryonic or postnatal lethal. These included genes in three major categories, although additional features and some overlap exist: 1) homeobox genes (*Hesx1, Lhx3, Lhx4, Prop1, Sox2,* and *Sox10*); 2) fibroblast growth factor pathways (*Dusp6, Fgf8, Fgfr1*—exon B variant, *Flrt3,* and *Hs6st1*); and 3) central nervous system and midline cranial defects with or without other anomalies (*Chd7, Dmxl2, Fezf1, Pnpla6, Polr3a, Polr3b, Smchd1, Stub1, Dmxl2,* and *Wdr11*); and as well as several others (*Pcsk1, Ndn).* For at least two genes, *Fgfr1* and *Wdr11*, although most are lethal, rarely some mice that reach adulthood are able to reproduce, but still exhibit infertility. Somewhat surprisingly, only 10/43 (23%) of the homozygotes manifested hypogonadotropic hypogonadism as the reproductive phenotype with or without other anomalies. These included: *Gnrh1, Gnrhr, Kiss1, Kiss1r, Lep, Lepr, Fgfr1, Prok2, Prokr2,* and *Lhb,* and *Sema3a.*

Of the remaining 20 homozygous mouse models, 1/43 (5%) did not have a reproductive specified (*Il17rd*); 3/43 (7%) were fertile (*Fgf17, Sema3e,* and *Spry4,*) and the remaining 7/43 (16%) had subfertility. For *Sox3,* subfertility was not specified if it affected both sexes. Subfertility in *Nsmf* KO mice is sexually dimorphic, being more severe in females than males.(Quaynor et al. 2015; Spilker et al. 2016) Hypofunction of three genes (*Tac3, Tacr3, Fshb*) resulted in fertile males, but subfertile females, while pathogenic variants in *Rnf216* and *Nr0b1* were associated with normal fertility in females, but subfertility in males (Table 4).

As detailed above, nearly half of the mouse models of hypogonadotropic hypogonadism manifested lethality in the homozygous state. If the heterozygotes for these 22 genes are analyzed, we observed the following: 12/22 (54%) were fertile, 5/22 (23%) were subfertile, and for 5/22 (23%), the reproductive potential was not available. Viable heterozygotes, such as *Hesx1* KO, did manifest signs of pituitary failure with hypogonadotropic hypogonadism.

Overall, most mouse models had relevance to the phenotype of nHH/KS even though nearly half were lethal. Of the 56 homozygous mouse models, 53% were lethal; 21% displayed nHH/KS; 16% had subfertility leaving 7% that were fertile and 5% for which the reproductive phenotype was not available. A high mortality among homozygous knockout/hypomorphic mice is not particularly surprising given the involvement of homeobox genes, the FGF pathway and CNS/midline defects. What is more surprising is that nearly one quarter of mouse models had pubertal development and were fertile or subfertile, suggesting some differences with the more severe human phenotype. Despite varying severity in phenotypes, very valuable scientific evidence regarding the intricacies of reproduction have been learned from these animal models

5.0 Inheritance Patterns of nHH/KS Genes & Genetic Counseling

The inheritance patterns of nHH/KS are shown in Table 2A for 44 genes recognized and given MIM numbers by OMIM. Only three genes—*ANOS1* (Franco et al. 1991; Legouis et al. 1991), *NR0BI,* and *SOX3* are X-linked recessive, and so should affect males. All of the rest are autosomal, which is interesting given the increased male to female ratio, which could indicate some bias of ascertainment of nHH/KS patients. Note that 22 are categorized as autosomal recessive and 21 are autosomal dominant. *HESX1* and *IL17RD* variants may either be inherited in an autosomal dominant or autosomal recessive fashion. Others could also be considered in two categories, such as *PROK2* (Cole et al. 2008; Cox et al. 2018), *PROKR2* (Dode and Rondard 2013), and *NSMF* (Xu et al. 2011), because monoallelic and biallelic variants have been described in these genes and not enough families have been characterized for segregation analysis. Interestingly, some of these heterozygous variants also coexist with a heterozygous variant in a second gene, suggesting the possibility of digenic disease (see below) in 2-10%. Ideally, families are best studied by constructing a three-generation pedigree.

5.1 X-Linked Recessive Forms of nHH/KS

There are three known X-linked recessive genes involved in nHH/KS—two associated with KS (*ANOS1* and *SOX3*) and one associated with nHH (*NR0B1*). Affected males have only one X chromosome—they are hemizygous. Affected males will pass this X-chromosome gene variant to all daughters, who will be carriers, but none of their sons will be affected. Females are typically unaffected in X-linked recessive disorders unless there they have a 45,X karyotype (very unlikely), variants are biallelic, or there is skewed X-inactivation. Normally X-inactivation is random in cells, but if there is preferential inactivation of the normal X

chromosome, the remaining chromosome with the pathogenic variant could result in a phenotype of nHH/KS. There is little compelling evidence that female carriers for *ANOS1, NR0B1,* or *SOX3* pathogenic variants manifest nHH/KS. One female with biallelic *ANOS1* variants manifested KS, but other variants in females have occurred in the presence of pathogenic variants in other genes (Shaw et al. 2011).

Carrier females of X-linked recessive diseases will transmit their abnormal X chromosome to half of their daughters (who will be carriers) and to half of their sons (who will be affected). For practical genetic counseling of apparent X-linked recessive families (affected males inherited via carrier females), *ANOS1* gene variants will be the most commonly dealt with X-linked recessive gene. In this instance, associated phenotypic findings can be very helpful. If anosmia/hyposmia, midline facial defects, cryptorchidism, unilateral renal agenesis, and/or synkinesia is present in a family with X-linked recessive inheritance, *ANOS1* is the likely culprit. If there is adrenal hypoplasia congenita (AHC) in the normosmic patient, then *NR0B1* will be more likely. If there is CPHD with or without intellectual disability, *SOX3* is more likely. The latter two are much less common.

5.2 Autosomal Recessive Forms of nHH/KS

There are at least 22 genes for which variants result in autosomal recessive disease (Table 2A). The affected individuals, who may be female or male, have biallelic variants which may be identical (homozygous) or different (compound heterozygous). Compound heterozygous pathogenic variants are very common for *GNRHR*.(Bhagavath et al. 2005; Francou et al. 2016) When homozygous variants for rare diseases are encountered, consanguinity should be suspected and ascertained. In autosomal recessive inheritance, both parents are nearly always heterozygous carriers so their recurrence risk is 25% for an affected child, 50% for an unaffected carrier, and 25% for a normal phenotype. Three of the most commonly involved autosomal recessive forms of nHH/KS are due to *GNRHR, TACR3,* or *KISS1R* pathogenic variants as there have been more than 20 families reported with *TACR3* and *KISS1R* variants, and more than 60 for *GNRHR*) (Maione et al. 2018).

Pathogenic variants in *LEP, LEPR, KISS1, PCSK1,* and *GNRH1* genes are uncommon, and each of these results in nHH in less than 1% of patients (Layman 2013b). In an autosomal recessive pedigree, other phenotypic features may assist in the diagnosis and counseling. Variants in *DMLX2* and *IL17RD* are commonly associated with deafness, while pathogenic variants in *FEZF1* result in KS, and others are associated with neurologic diseases (*PNPLA6, POLR3A, POLR3B,* and *RNF216* (Table 3).

If the individual with an nHH autosomal recessive pedigree is counseled for future fertility, most commonly because of *GNRHR* or *TACR3* variants, the recurrence risk for that individual is very low. The affected individual would have to reproduce with another carrier, which would be very unlikely unless there is consanguinity. If we use a carrier rate of 1/100, so the chance of an affected child would be 1 (the probability for the affected individual to be a carrier) X 1/100 (background carrier rate) X 1/2 chance of the background carrier to pass on the variant = 1/200. In reality, the carrier frequency is much lower than that, so the risk is very small.

There are some special cases of gene variants affecting pituitary function that result in autosomal recessive disease. If there is CPHD in family members, then *PROP1, LHX3,* and *HESX1* need to be considered. *HESX1* variants can also be inherited in an autosomal dominant fashion. If hypogonadism is present, but FSH is very low and LH is elevated, *FSHB* pathogenic variants could be causative (Matthews and Chatterjee 1997; Layman et al. 1997; Layman et al. 2002). Similarly, if LH is low and FSH is elevated or normal, *LHB* variants should be sought (Lofrano-Porto et al. 2007; Weiss et al. 1992). For some of the other genes in Table 2, not enough families have been described to be useful for practical genetic counseling.

5.3 Autosomal Dominant Forms of nHH/KS

Autosomal dominant diseases represent the most challenging to detect and counsel because of reduced penetrance and variable expressivity. The most commonly encountered autosomal dominant inherited gene variants are in *FGFR1, CHD7, PROKR2,* and *WDR11* (Maione et al. 2018; Boehm et al. 2015). Variants in *SOX10* are common among those patients with hearing loss (Pingault et al. 2013). Affected individuals may be either males or females with heterozygous pathogenic variants. Heterozygous variants may either cause disease because of haploinsufficiency, in which ~50% reduction in protein is not able to adequately sustain normal function, or by a dominant negative mechanism, in which the variant interferes with the function of the wild type allele. *CHD7* variants commonly exhibit haploinsufficiency, particularly truncating variants. However, in depth studies of *PROKR2* variants, which are commonly missense variants, have revealed that some are consistent with haploinsufficiency, but others support a dominant negative effect (Cox et al. 2018). Dominant negative variants typically affect proteins that form dimers (such as *PROKR2*) or multimeric proteins such as collagen.

Autosomal dominant traits have a 50:50 chance of transmission to a son or daughter. Variants may be inherited from a parent, but they may also be absent in both parents (*de novo*) or there may be gonadal mosaicism, in which pathogenic variants are present in some germ cells, but not in peripheral blood DNA. For most autosomal dominant diseases, the recurrence risk is ~ 2-4% if gonadal mosaicism is present. Gonadal mosaicism has been reported for *FGFR1* variants.(Sato et al. 2006) In a recent study involving whole exome sequencing in 60 nHH/KS trios (patient and parents), postzygotic mosaicism for two *FGFR1* variants and one *CHD7* variant were identified in 3 of 10 patients when multiple tissues were studied. These findings reinforce available information for genetic counseling (Acierno et al. 2020). However, once the individual has a heterozygous variant, it will behave in an autosomal dominant fashion with a 50% risk for each pregnancy.

The phenotypic effects of autosomal dominantly inherited pathogenic variants may vary from mild to severe (variable expressivity). It is possible, for example, that a female with a *CHD7* pathogenic variant has KS and mild hearing loss, but she could have a more severely affected child with KS, congenital heart disease, midline facial clefting, and severe deafness. What complicates this situation even more is that not all individuals with the pathogenic variant exhibit any phenotypic effects—this is reduced penetrance. Yet they are still at the same risk of transmitting the variant to 50% of their offspring. These features always make autosomal dominant disease difficult to predict future risk.

When an apparently unaffected person has a pathogenic variant in one of the autosomal dominant genes, it is perhaps safest to counsel up to a 50% recurrence risk to a child. This same advice could be provided for an affected individual with nHH/KS who has not yet been studied for pathogenic variants in any nHH/KS genes. For two genes (*HESX1* and *IL17RD*), for which variants may be inherited as autosomal dominant or autosomal recessive, performing a pedigree and testing available family members would provide the most important information. If that is not possible, erring on the side of autosomal dominant with a 50% recurrence risk seems prudent. This is also true for *PROKR2* and *PROK2*, as some families may have autosomal recessive appearing pedigrees, while others have autosomal dominant ones, which manifest variable expressivity and reduced penetrance (Dode et al. 2006; Cox et al. 2018).

6.0 Digenic nHH/KS

Digenic and oligogenic variants have been increasingly reported by a number of investigators, but confirming digenic inheritance is extremely difficult to prove and it requires a combination of human families (which are usually rare), animal models, and cell-based studies (Schaffer 2013). Digenicity for KS was first suggested for *PROKR2* and *ANOS1* variants, but variants were not studied *in vitro (Dode et al. 2006).* Digenic families in which variants for both genes underwent *in vitro* analyses with familial segregation were reported in 2007 for *FGFR1/NSMF* and *FGFR1/GNRHR* (Pitteloud, Quinton, et al. 2007). Subsequently, many additional cases have been reported. In a compilation of cases from the literature, we identified 67 probands who had variants in two or more of the 44 nHH/KS genes recognized by OMIM (Table 5, which includes the reference and PMID number for each) (Dode et al. 2006; Pitteloud, Quinton, et al. 2007; Cole et al. 2008; Falardeau et al. 2008; Canto et al. 2009; Sykiotis et al. 2010; Sarfati et al. 2010; Quaynor et al. 2011; Beneduzzi et al. 2011; Miraoui et al. 2013; Margolin et al. 2013; Xu et al. 2017; McCormack et al. 2017; Goncalves et al. 2017; Quaynor et al. 2016; Tornberg et al. 2011; Zhou et al. 2018).

From these studies, we identified eight unrelated individuals with digenic variants in nHH/KS supported by *in vitro* confirmation for both variants and 20 probands with supportive *in vitro* analysis for one of the two genes (the other was not studied), and 39 had digenic variants where neither was studied—i.ie., VUS. These latter 39 digenic variants will not be discussed further. All 67 digenic variant combinations are shown in Table 4. For these first two groups of 8 and 20 probands, 12 genes were involved (Table 2A, 2B; Table 5)—*ANOS1, SEMA3A, GNRHR, RNF216, TACR3, FGF8, FGFR1, HS6ST1, NSMF, PROK2, PROKR2,* and *WDR11*.

6.1 Eight Digenic Cases Where Both Variants Were Studied In Vitro

Eight probands had digenic variants that were deleterious *in vitro*. Six genes were involved—five autosomal dominant (*FGFR1, PROKR2, NSMF, SEMA3A*, and *WDR11*) and one autosomal recessive (*GNRHR*) gene. Four cases involved *FGFR1* and four involved *PROKR2* variants, all of which were heterozygous. The second gene variant along with *FGFR1* involved either heterozygous *NSMF* (n = 1), biallelic *GNRHR* (n = 2), or heterozygous *GNRHR* variants. For *PROKR2* variants, the second gene consisted of heterozygous variants in either *NSMF* (n =1), *SEMA3A* (n = 2), or *WDR11* (n = 1). In seven of the eight cases, the variants in either gene could result in the phenotype without involvement of the other gene. In one case, a heterozygous *GNRHR* variant should not be sufficient to cause disease because it is autosomal recessive.

For five of these cases, pedigrees were reported, but for the three *SEMA3A/PROKR2* digenic variants, only the proband was genotyped. As can be seen in Figure 3 for cases 1 (*FGFR1/NSMF*), 2 (*GNRHR/FGFR1*) and 8 (*PROKR2/WDR11*), digenic inheritance is supported as there are individuals with both gene variants who are affected, whereas persons with one gene variant may either be affected or unaffected. For case 3 and 4, digenicity was more difficult to support since none of the unaffected family members were genotyped. No animal models were reported for any of these cases. Therefore, support for digenicity in three of these cases comes from two of the requirements: human pedigrees and cell-based *in vitro* studies, but not animal studies. It should also be pointed out that only one family for each digenic pair was identified. It is interesting to note that 15 of the 16 genotypes in these individuals are sufficient to cause nHH/KS without a variant in a second gene.

6.2 Twenty Digenic Cases Where Only One Variant Was Studied In Vitro

The next best evidence for digenicity in humans comes from 20 gene pairs possessing variants, in which one was confirmed *in vitro* (Table 5). Only the gene variants in the digenic pair that were studied *in vitro* were counted as genes participating in digenicity. All 20 gene variants are sufficient to cause disease without a second gene. For the variants confirmed *in vitro*, *FGFR1* was once again the most common gene involved (n = 5), followed by *ANOS1* (n = 4), and then *WDR11, HS6ST1, SEMA3A*, and *NSMF*, found in two probands each. *PROKR2, FGF8,* and *GNRHR* were each represented once. All variants confirmed *in vitro* were heterozygous except *GNRHR* variants, which were biallelic. For the five cases of confirmed *FGFR1* variants, *FGFR1* variants are sufficient to explain the phenotype since *FGFR1* variants can cause nHH or KS. For the one female with KS (#27 in Table 5) who had heterozygous *FGFR1* and *TACR3* variants, it appears likely that *FGFR1* was causative. A heterozygous *TACR3* variant by itself should not result in the KS phenotype since it is autosomal recessive, and *TACR3* variants also cause nHH rather than KS.

The four cases of *ANOS1* pathogenic variants confirmed by *in vitro* studies occurred only in males with KS, and so *ANOS1* by itself could explain the phenotype. Two of the males also had heterozygous *PROKR2* VUS and two had heterozygous *TACR3* VUS. The second gene variant for the ten probands involving *WDR11, HS6ST1, SEMA3A, NSMF, GNRHR,* and *PROKR2* were also VUS (not studied *in vitro*), but if found to be causative, the other gene variant could potentially cause the disease by itself in 8 of 10 cases. For two cases, a heterozygous *GNRHR* variant should not be causative by itself, and the female with nHH who had biallelic *GNRHR* variants, also had a heterozygous *ANOS1*, which should not cause nHH in a female. Therefore, these monoallelic variants could potentially be responsible for the observed phenotype in all cases without any contribution from another gene!

Little can be ascertained from the 39 cases with VUS since they have not been studied *in vitro*. It should be noted that cases 57-67 were part of a study in which only males were studied (Zhou et al. 2018). Most of the cases from Table 5 were studied with known genes at the time, which has increased over time so that digenicity could be underestimated. To get a truly unbiased ascertainment of digenic inheritance, all genes would need to

be studied. However, whole exome sequencing and whole genome sequencing have only been applied more recently, which likely will increase the detection rate of digenic/oligogenic variants in nHH/KS.

Just because digenic (or oligogenic in a few) variants are present, this does not prove that both genes are involved in the phenotype. This is a much understudied area of hypogonadotropic hypogonadism genetics. As published by Schaffer (Schaffer 2013), digenic inheritance is extremely difficult to prove and it requires a combination of human families (which unfortunately are rare), animal models, and cellular models. Although there are 28 different genes reported in digenic inheritance in humans, there is very little proof of causation with a few exceptions. We only found three mouse models supporting digenicity. In the double *Fgf8* +/-//*Fgfr1*+/- mouse, but not *Fgf8/Fgfr3* or *Fgfr1/Fgfr3* double heterozygotes, GnRH neuron migration was more adversely impaired with the double heterozygote compared with the single heterozygote (Chung et al. 2010). Two *FGF8/FGR1* heterozygous digenic pathogenic variant have also been described in humans (cases 10 and 11 in Table 5), providing further documentation for digenic diease (Falardeau et al. 2008). Therefore, strongest support for digenicity comes from *FGF8/FGFR1* genes since all three criteria for digenicity have been supported, although only two families have been identified.

Another example supporting digenicity in an animal model is for *RNF216* and *OTUD4*. Homozygous pathogenic variants in both genes were identified in one consanguineous family with nHH and ataxia (Gordon-Holmes syndrome) (Margolin et al. 2013). When these genes were knocked down in zebrafish, a greater impairment of optic tectum size was noted compared with each gene separately (Margolin et al. 2013). *OTUD4* is currently a GUS, because unlike *RNF216* where additional variants in GHS were found, no additional *OTUD4* variants have been reported. Nevertheless, there is support from the animal model for digenicity. Although the *Kiss1/Kiss1r* double KO mouse appears to have a more severe phenotype than either alone (Chan et al. 2011), we were unable to find corresponding pathogenic variants in both genes in a human with nHH.

Mechanisms of how variants in two or more genes result in disease include: 1) chance alone; 2) digenicity; 3) synergistic heterozygosity, or 4) modifier gene effects (Schaffer 2013). Digenicity refers to the interaction of two nonhomologous genes, either monoallelic or biallelic. In true digenic disease, some individuals with variants in one gene will manifest the phenotype, while others do not; however, those with variants in both genes will be affected, and likely be more severely affected than with either gene alone. Synergistic heterozygosity is defined as variants in two genes, either affecting intra- or interpathway interactions, which are not sufficient to result in disease individually, but that are causative in combination (Vockley et al. 2000; Schuler et al. 2005; Phillips et al. 2008). They do not have to be involved directly in the same pathway, but could contribute to a common pathway such as GnRH neuron migration, GnRH secretion, etc. Synergistic heterozygosity was described in biochemical genetics in which heterozygous variants to two genes in the same biochemical pathway resulted in disease, which would normally only occur if either gene had biallelic variants in an autosomal recessive condition (Vockley et al. 2000). Lastly, the second gene involved could be a modifier gene, in which a gene at a different locus modifies the phenotype—either making it worse or milder. A classic example is how *MBL2* and *TGFB1* may modify the cystic fibrosis phenotype in affected individuals with *CFTR* pathogenic variants (Bartlett et al. 2009).

In depth studies of digenicity are extremely important in understanding the pathophysiology of nHH/KS and could profoundly affect genetic counseling of affected patients. At this time, it appears that 12 genes demonstrated some evidence for digenicity, most of which are autosomal dominant and can cause either nHH or KS (Table 2, Table 5). *FGF8/FGFR1* offers the strongest support for all three criteria for digenicity, while human studies for *FGFR1/NSMF*, *FGFR1/GNRHR*, and *PROKR2/WDR11* and animal model findings for *RNF216/OTUD4* and *KISS1/KISS1R* provide support for digenicity. It is clear that future studies are needed to clarify and confirm the issue of digenicity in nHH/KS.

7.0 Clinical Genetic Diagnosis of nHH/KS

The genetic basis for nHH/KS has been elucidated for approximately half of patients. Karyotype is generally not helpful since no consistent abnormalities have been reported. In one study of 76 patients, ~4% had either a balanced translocation or a pericentric inversion, but they are not known to be causative without further analysis (Bhagavath et al. 2006). One of these translocations was instrumental in identifying the *WDR11* gene as a causative nHH/KS gene (Kim et al. 2010). The use of chromosomal microarrays (CMA) will not usually be helpful since single nucleotide variants and small indels constitute most of the known etiologies. However,

when intellectual disability or another apparently unrelated phenotype accompanies nHH/KS, CMA may be utilized to demonstrate a contiguous gene deletion syndrome. One example is an Xp22.3 deletion encompassing *ANOS1* causing KS and *STS*, which causes ichthyosis (Maya-Nunez et al. 1998). Contiguous gene deletions of chromosome 8p11-p12, which includes *FGFR1,* have also been reported in patients with KS and either congenital anemia due to spherocytosis or visual impairment (Dode et al. 2003). DNA sequencing of candidate genes constitutes the current state of diagnosis for most patients.

Given the large numbers of implicated genes for nHH/KS and possible oligogenic inheritance, how should the clinician proceed to perform genetic testing? A minimalist approach might be to perform DNA sequencing for the most common genes involved. Pathogenic variants in *FGFR1* (10%) and *CHD7* (6%) are common in both nHH and KS, so one argument could be to test for these two in nHH and add *GNRHR* (4-5%) and *TACR3* (5-6%). This would give a detection rate of ~25% (Layman 2013a). For KS, testing *FGFR1, CHD7,* and *ANOS1* (if a male) would yield pickup rates of 20% in males and 16% in females (Layman 2013a).

Testing could be targeted depending upon the associated anomalies, such as synkinesia (*FGFR1, ANOS1*), deafness (*SOX10, CHD7, IL17RD, DMXL2*), ataxia (*RNF216, STUB1, PNPLA6*), midline facial defects (*CHD7* and FGF pathway genes), or septo-optic dysplasia (*HESX1* and *SOX2*). Others are shown in Table 3. Testing could be based upon the sense of smell. As shown in Table 2B, variants in 9 genes result in KS; 21 result in nHH; and 12 result in either nHH or KS. Isolated deficiencies of gonadotropins will be dictated by serum FSH and LH levels. If the patient is anosmic/hyposmic, it would be reasonable to sequence these 21 (9 KS; 12 nHH/KS) genes. If the patient is normosmic, then sequencing of 33 genes (21 nHH; 12 nHH/KS) could be performed

. Inheritance within the family could also provide clues if there are several affected individuals, although some gene variants can be inherited as dominant or recessive (*HESX1, IL17RD, PROK2, PROKR2, NSMF*). As can be seen in Table 2A, about half of the genes with variants are inherited as autosomal recessive and half are autosomal dominant. Only 3/44 (7%) are X-linked recessive. Genes participating (but not proven) in digenic/oligogenic inheritance are also indicated (in bold) in Tables 2A and 2B. Although important to consider these genes, the role of digenic causation is largely unknown and needs to be studied before more specific guidelines can be generated. Careful evaluation of the genotypes and phenotypes of affected and unaffected family members is required. In true digenic disease, patients with pathogenic variants in one gene may or may not be affected, while those with pathogenic variants in two genes should be affected (Schaffer 2013).

Right now, targeted next generation sequencing panels of all 44 genes provides the most practical method of DNA diagnosis, but variants should be confirmed by Sanger sequencing. It is likely in the near future, the 14 additional GUS discussed above could be added to the panel, as well as other newly discovered genes. Intragenic deletions are not commonly reported in nHH/KS, but a reflex deletion analysis by MLPA or qPCR could be performed if pathogenic variants are absent, but certain specific genes are suspected. Large gene deletions are not commonly described for most nHH/KS genes, but have been identified for *ANOS1* and *FGFR1*. When patients manifest intellectual disability and additional somatic anomalies not commonly associated with nHH/KS, contiguous gene deletion syndromes could be present so that microarrays could be considered.

Finally, whole exome sequencing (WES) or whole genome sequencing (WGS) could be performed. It must be realized that particularly with WES, not all bases of every gene may be interrogated (or captured in the library preparation). This technique could be considered when the 44 gene panel does not yield any pathogenic variants. Whole genome sequencing allows a more unbiased approach and may be useful for copy number of some genes/regions, but many variants of undetermined significance will be identified. Currently WGS is probably best reserved for research purposes, but it will likely have increasing usage as the analysis is better understood and the cost decreases. Improved sequencing methods, and in particular, more robust bioinformatics analysis of big data (Suwinski et al. 2019), as well as improved detection of structural variants by methods such as genome optical mapping (Chaisson et al. 2019), will facilitate more complete genetic analysis of these patients.

7.1 Fertility Considerations

When patients with nHH/KS contemplate pregnancy, it is important to provide them with information regarding their future fertility through a multidisciplinary approach. This may include referrals to mental health professionals, geneticists/genetic counselors, and fertility specialists. As discussed above, genetic counseling should include an explanation of the inheritance pattern of the involved gene variant and the likelihood of transmitting the disease to offspring. This is particularly important for autosomal dominant genes since it is complicated by both variable expressivity and reduced penetrance, and there is a higher likelihood of transmission to offspring.

Fertility can often be restored in males using pulsatile GnRH or subcutaneous gonadotropins 2-3 times/week. Early studies showed ~88% success in males treated with gonadotropin therapy.(Crowley and Whitcomb 1990) Findings from a meta-analysis demonstrated an overall success rate of~ 75% of achieving spermatogenesis, with a mean sperm concentration obtained of ~6 million/mL for gonadotropins and ~4 million/mL for GnRH therapy (Rastrelli et al. 2014). This treatment for males may take 6-12 months or even longer depending upon several predictive factors. Specific recommendations based upon the gene variant involved are not currently available due to small numbers of identified individuals seeking fertility treatment. Poor prognostic factors for males include small testes (<4cc), cryptorchidism, and low inhibin B, which more commonly occur in males with *ANOS1* pathogenic variants (Salenave et al. 2008). Important for treatment is the evaluation of the female partner for tubal patency and ovulation.

Females may be treated with subcutaneous gonadotropins, usually both FSH and LH, or GnRH. Successful ovulation induction occurs in 60-80% of the cases with a pregnancy rate of 20-50% if there is no male factor (Pandurangi, Tamizharasi, and Reddy 2015). Even in the presence of compound heterozygous *GNRHR* pathogenic variants, pulsatile GnRH administration was capable to overcome the receptor defects and induce ovulation and successful pregnancies (Seminara et al. 2000). As more patients with nHH/KS undergo genetic analysis, gene-specific fertility rates could become available for counseling purposes. For now, patients should be informed that GnRH and gonadotropin therapy is usually successful depending upon age, the phenotypic features mentioned, and associated partner factors.

Gene-specific effects have been studied in the ~10% of patients with nHH/KS who experienced spontaneous reversal (Raivio et al. 2007). One group (Sidhoum et al. 2014) showed spontaneous reversal in 22% of patients with *FGFR1, PROKR2*, and *GNRHR* pathogenic variants, but the rate of reversal was not statistically different amongst these genes. Notably, no patients with *ANOS1* variants experienced reversal (Sidhoum et al. 2014). However, as discussed above, patients with pathogenic variants in the tachykinin pathway (*TAC3* and *TACR3)* have a much better prognosis for spontaneous reversal and fertility.

8.0 Conclusion

In the past 35 years, great strides have been made in the identification and characterization of genes involved in nHH/KS with and without other associated anomalies. Currently, 44 likely causative genes have variants in about 50% of nHH/KS patients, which are complemented by 43 mouse models. It is likely new genes will be characterized, perhaps facilitated by chromosomal rearrangements (Supplemental Table 1). With the advent of WES and WGS, a better characterization of digenic variants will increase our understanding of the role in oligogenicity in these disorders. It must be remembered that pituitary deficiency due to variants homeobox genes causing combined pituitary deficiency should be considered where appropriate. Human and animal models will aid in the clarification of the role of nHH/KS genes in the diagnosis of patients with pubertal disorders and will enhance our understanding of normal puberty.

References

Abreu, A. P., A. Dauber, D. B. Macedo, S. D. Noel, V. N. Brito, J. C. Gill, P. Cukier, I. R. Thompson, V. M. Navarro, P. C. Gagliardi, T. Rodrigues, C. Kochi, C. A. Longui, D. Beckers, F. de Zegher, L. R. Montenegro, B. B. Mendonca, R. S. Carroll, J. N. Hirschhorn, A. C. Latronico, and U. B. Kaiser. 2013. 'Central precocious puberty caused by mutations in the imprinted gene MKRN3', *N Engl J Med*, 368: 2467-75.

Acierno, J. S., C. Xu, G. E. Papadakis, N. J. Niederlander, J. D. Rademaker, J. Meylan, A. Messina, Z. Kolesinska, R. Quinton, M. Lang-Muritano, D. Bartholdi, I. Halperin, C. De Geyter, J. Bouligand, L. Bartoloni, J. Young, F. A. Santoni, and N. Pitteloud. 2020. 'Pathogenic mosaic variants in congenital hypogonadotropic hypogonadism', *Genet Med*, 22: 1759-67.

Amato, L. G. L., L. R. Montenegro, A. M. Lerario, A. A. L. Jorge, G. Guerra Junior, C. Schnoll, A. C. Renck, E. B. Trarbach, E. M. F. Costa, B. B. Mendonca, A. C. Latronico, and L. F. G. Silveira. 2019. 'New genetic findings in a large cohort of congenital hypogonadotropic hypogonadism', *Eur J Endocrinol*, 181: 103-19.

Barraud, S., B. Delemer, C. Poirsier-Violle, J. Bouligand, J. C. Merol, F. Grange, B. Higel-Chaufour, B. Decoudier, M. Zalzali, A. A. Dwyer, J. S. Acierno, N. Pitteloud, R. P. Millar, and J. Young. 2021. 'Congenital Hypogonadotropic Hypogonadism with Anosmia and Gorlin Features Caused by a PTCH1 Mutation Reveals a New Candidate Gene for Kallmann Syndrome', *Neuroendocrinology*, 111: 99-114.

Bartlett, J. R., K. J. Friedman, S. C. Ling, R. G. Pace, S. C. Bell, B. Bourke, G. Castaldo, C. Castellani, M. Cipolli, C. Colombo, J. L. Colombo, D. Debray, A. Fernandez, F. Lacaille, M. Macek, Jr., M. Rowland, F. Salvatore, C. J. Taylor, C. Wainwright, M. Wilschanski, D. Zemkova, W. B. Hannah, M. J. Phillips, M. Corey, J. Zielenski, R. Dorfman, Y. Wang, F. Zou, L. M. Silverman, M. L. Drumm, F. A. Wright, E. M. Lange, P. R. Durie, M. R. Knowles, and Group Gene Modifier Study. 2009. 'Genetic modifiers of liver disease in cystic fibrosis', *JAMA*, 302: 1076-83.

Beneduzzi, D., A. K. Iyer, E. B. Trarbach, A. P. Silveira-Neto, L. G. Silveira, C. Tusset, K. Yip, B. B. Mendonca, P. L. Mellon, and A. C. Latronico. 2011. 'Mutational analysis of the necdin gene in patients with congenital isolated hypogonadotropic hypogonadism', *Eur J Endocrinol*, 165: 145-50.

Bhagavath, B., M. Ozata, I. C. Ozdemir, E. Bolu, D. P. Bick, R. J. Sherins, and L. C. Layman. 2005. 'The prevalence of gonadotropin-releasing hormone receptor mutations in a large cohort of patients with hypogonadotropic hypogonadism', *Fertil Steril*, 84: 951-7.

Bhagavath, B., R. H. Podolsky, M. Ozata, E. Bolu, D. P. Bick, A. Kulharya, R. J. Sherins, and L. C. Layman. 2006. 'Clinical and molecular characterization of a large sample of patients with hypogonadotropic hypogonadism', *Fertil Steril*, 85: 706-13.

Bick, D., B. Franco, R.S. Sherins, B. Heye, L. Pike, J. Crawford, A. Maddalena, B. Incerti, A. Pragliola, T. Meitinger, and A. Ballabio. 1992. 'Intragenic deletion of the KALIG-1 gene in Kallmann's syndrome', *N Engl J Med*, 326: 1752-55.

Boehm, U., P. M. Bouloux, M. T. Dattani, N. de Roux, C. Dode, L. Dunkel, A. A. Dwyer, P. Giacobini, J. P. Hardelin, A. Juul, M. Maghnie, N. Pitteloud, V. Prevot, T. Raivio, M. Tena-Sempere, R. Quinton, and J. Young. 2015. 'Expert consensus document: European Consensus Statement on congenital hypogonadotropic hypogonadism--pathogenesis, diagnosis and treatment', *Nat Rev Endocrinol*, 11: 547-64.

Bouligand, J., C. Ghervan, J. A. Tello, S. Brailly-Tabard, S. Salenave, P. Chanson, M. Lombes, R. P. Millar, A. Guiochon-Mantel, and J. Young. 2009. 'Isolated familial hypogonadotropic hypogonadism and a GNRH1 mutation', *N Engl J Med*, 360: 2742-8.

Burke, M. C., P. A. Letts, S. J. Krajewski, and N. E. Rance. 2006. 'Coexpression of dynorphin and neurokinin B immunoreactivity in the rat hypothalamus: Morphologic evidence of interrelated function within the arcuate nucleus', *J Comp Neurol*, 498: 712-26.

Canto, P., P. Munguia, D. Soderlund, J. J. Castro, and J. P. Mendez. 2009. 'Genetic analysis in patients with Kallmann syndrome: coexistence of mutations in prokineticin receptor 2 and KAL1', *J Androl*, 30: 41-5.

Caronia, L. M., C. Martin, C. K. Welt, G. P. Sykiotis, R. Quinton, A. Thambundit, M. Avbelj, S. Dhruvakumar, L. Plummer, V. A. Hughes, S. B. Seminara, P. A. Boepple, Y. Sidis, W. F. Crowley, Jr., K. A. Martin, J.

E. Hall, and N. Pitteloud. 2011. 'A genetic basis for functional hypothalamic amenorrhea', *N Engl J Med*, 364: 215-25.

Cassatella, D., S. R. Howard, J. S. Acierno, C. Xu, G. E. Papadakis, F. A. Santoni, A. A. Dwyer, S. Santini, G. P. Sykiotis, C. Chambion, J. Meylan, L. Marino, L. Favre, J. Li, X. Liu, J. Zhang, P. M. Bouloux, C. Geyter, A. Paepe, W. S. Dhillo, J. M. Ferrara, M. Hauschild, M. Lang-Muritano, J. R. Lemke, C. Fluck, A. Nemeth, F. Phan-Hug, D. Pignatelli, V. Popovic, S. Pekic, R. Quinton, G. Szinnai, D. l'Allemand, D. Konrad, S. Sharif, O. T. Iyidir, B. J. Stevenson, H. Yang, L. Dunkel, and N. Pitteloud. 2018. 'Congenital hypogonadotropic hypogonadism and constitutional delay of growth and puberty have distinct genetic architectures', *Eur J Endocrinol*, 178: 377-88.

Chaisson, M. J. P., A. D. Sanders, X. Zhao, A. Malhotra, D. Porubsky, T. Rausch, E. J. Gardner, O. L. Rodriguez, L. Guo, R. L. Collins, X. Fan, J. Wen, R. E. Handsaker, S. Fairley, Z. N. Kronenberg, X. Kong, F. Hormozdiari, D. Lee, A. M. Wenger, A. R. Hastie, D. Antaki, T. Anantharaman, P. A. Audano, H. Brand, S. Cantsilieris, H. Cao, E. Cerveira, C. Chen, X. Chen, C. S. Chin, Z. Chong, N. T. Chuang, C. C. Lambert, D. M. Church, L. Clarke, A. Farrell, J. Flores, T. Galeev, D. U. Gorkin, M. Gujral, V. Guryev, W. H. Heaton, J. Korlach, S. Kumar, J. Y. Kwon, E. T. Lam, J. E. Lee, J. Lee, W. P. Lee, S. P. Lee, S. Li, P. Marks, K. Viaud-Martinez, S. Meiers, K. M. Munson, F. C. P. Navarro, B. J. Nelson, C. Nodzak, A. Noor, S. Kyriazopoulou-Panagiotopoulou, A. W. C. Pang, Y. Qiu, G. Rosanio, M. Ryan, A. Stutz, D. C. J. Spierings, A. Ward, A. E. Welch, M. Xiao, W. Xu, C. Zhang, Q. Zhu, X. Zheng-Bradley, E. Lowy, S. Yakneen, S. McCarroll, G. Jun, L. Ding, C. L. Koh, B. Ren, P. Flicek, K. Chen, M. B. Gerstein, P. Y. Kwok, P. M. Lansdorp, G. T. Marth, J. Sebat, X. Shi, A. Bashir, K. Ye, S. E. Devine, M. E. Talkowski, R. E. Mills, T. Marschall, J. O. Korbel, E. E. Eichler, and C. Lee. 2019. 'Multi-platform discovery of haplotype-resolved structural variation in human genomes', *Nat Commun*, 10: 1784.

Chan, Y. M., S. Broder-Fingert, S. Paraschos, R. Lapatto, M. Au, V. Hughes, S. D. Bianco, L. Min, L. Plummer, F. Cerrato, A. De Guillebon, I. H. Wu, F. Wahab, A. Dwyer, S. Kirsch, R. Quinton, T. Cheetham, M. Ozata, S. Ten, J. P. Chanoine, N. Pitteloud, K. A. Martin, R. Schiffmann, H. J. Van der Kamp, S. Nader, J. E. Hall, U. B. Kaiser, and S. B. Seminara. 2011. 'GnRH-deficient phenotypes in humans and mice with heterozygous variants in KISS1/Kiss1', *J Clin Endocrinol Metab*, 96: E1771-81.

Chan, Y. M., A. de Guillebon, M. Lang-Muritano, L. Plummer, F. Cerrato, S. Tsiaras, A. Gaspert, H. B. Lavoie, C. H. Wu, W. F. Crowley, Jr., J. K. Amory, N. Pitteloud, and S. B. Seminara. 2009. 'GNRH1 mutations in patients with idiopathic hypogonadotropic hypogonadism', *Proc Natl Acad Sci U S A*.

Chew, S., R. Balasubramanian, W. M. Chan, P. B. Kang, C. Andrews, B. D. Webb, S. E. MacKinnon, D. T. Oystreck, J. Rankin, T. O. Crawford, M. Geraghty, S. L. Pomeroy, W. F. Crowley, Jr., E. W. Jabs, D. G. Hunter, P. E. Grant, and E. C. Engle. 2013. 'A novel syndrome caused by the E410K amino acid substitution in the neuronal beta-tubulin isotype 3', *Brain*, 136: 522-35.

Cho, H. J., Y. Shan, N. C. Whittington, and S. Wray. 2019. 'Nasal Placode Development, GnRH Neuronal Migration and Kallmann Syndrome', *Front Cell Dev Biol*, 7: 121.

Chung, W. C., T. A. Matthews, B. K. Tata, and P. S. Tsai. 2010. 'Compound deficiencies in multiple fibroblast growth factor signalling components differentially impact the murine gonadotrophin-releasing hormone system', *J Neuroendocrinol*, 22: 944-50.

Clement, K., C. Vaisse, N. Lahlou, S. Cabrol, V. Pelloux, D. Cassuto, M. Gourmelen, C. Dina, J. Chambaz, J.-M. Lacorte, A. Basdevant, P. Bougneres, Y. Lebouc, P. Froguel, and B. Guy-Grand. 1998. 'A mutation in the human leptin receptor gene causes obesity and pituitary dysfunction', *Nature*, 392: 398-401.

Cole, L. W., Y. Sidis, C. Zhang, R. Quinton, L. Plummer, D. Pignatelli, V. A. Hughes, A. A. Dwyer, T. Raivio, F. J. Hayes, S. B. Seminara, C. Huot, N. Alos, P. Speiser, A. Takeshita, G. Van Vliet, S. Pearce, W. F. Crowley, Jr., Q. Y. Zhou, and N. Pitteloud. 2008. 'Mutations in Prokineticin 2 (PROK2) and PROK2 Receptor (PROKR2) in Human Gonadotrophin-Releasing Hormone Deficiency: Molecular Genetics and Clinical Spectrum', *J Clin Endocrinol Metab*.

Cox, K. H., L. M. B. Oliveira, L. Plummer, B. Corbin, T. Gardella, R. Balasubramanian, and W. F. Crowley. 2018. 'Modeling mutant/wild-type interactions to ascertain pathogenicity of PROKR2 missense variants in patients with isolated GnRH deficiency', *Hum Mol Genet*, 27: 338-50.

Crowley, W. F., Jr., M. Filicori, D. I. Spratt, and N. F. Santoro. 1985. 'The physiology of gonadotropin-releasing hormone (GnRH) secretion in men and women', *Recent Prog Horm Res*, 41: 473-531.

Crowley, W. F., and R. W. Whitcomb. 1990. 'Gonadotropin-releasing hormone deficiency in men: diagnosis and treatment with exogenous gonadotropin-releasing hormone', *Am J Obstet Gynecol*, 163: 1752-8.

Daoud, H., M. Tetreault, W. Gibson, K. Guerrero, A. Cohen, J. Gburek-Augustat, M. Synofzik, B. Brais, C. A. Stevens, R. Sanchez-Carpintero, C. Goizet, S. Naidu, A. Vanderver, and G. Bernard. 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: 194-7.

Dattani, M.T., J.-P. Martinez-Barbera, P.Q. Thomas, J.M. Brickman, R. Gupta, I.-L. Martensson, H. Toresson, M. Fox, J.K.H. Wales, P.C. Hindmarsh, S. Krauss, R.S.P. Beddington, and I.C.A.F. Robinson. 1998. 'Mutations in the homeobox gene HESX1/Hesx1 associated with septo-optic dysplasia in human and mouse', *Nat Genet*, 19: 125-33.

Day, F. R., K. S. Ruth, D. J. Thompson, K. L. Lunetta, N. Pervjakova, D. I. Chasman, L. Stolk, H. K. Finucane, P. Sulem, B. Bulik-Sullivan, T. Esko, A. D. Johnson, C. E. Elks, N. Franceschini, C. He, E. Altmaier, J. A. Brody, L. L. Franke, J. E. Huffman, M. F. Keller, P. F. McArdle, T. Nutile, E. Porcu, A. Robino, L. M. Rose, U. M. Schick, J. A. Smith, A. Teumer, M. Traglia, D. Vuckovic, J. Yao, W. Zhao, E. Albrecht, N. Amin, T. Corre, J. J. Hottenga, M. Mangino, A. V. Smith, T. Tanaka, G. Abecasis, I. L. Andrulis, H. Anton-Culver, A. C. Antoniou, V. Arndt, A. M. Arnold, C. Barbieri, M. W. Beckmann, A. Beeghly-Fadiel, J. Benitez, L. Bernstein, S. J. Bielinski, C. Blomqvist, E. Boerwinkle, N. V. Bogdanova, S. E. Bojesen, M. K. Bolla, A. L. Borresen-Dale, T. S. Boutin, H. Brauch, H. Brenner, T. Bruning, B. Burwinkel, A. Campbell, H. Campbell, S. J. Chanock, J. R. Chapman, Y. I. Chen, G. Chenevix-Trench, F. J. Couch, A. D. Coviello, A. Cox, K. Czene, H. Darabi, I. De Vivo, E. W. Demerath, J. Dennis, P. Devilee, T. Dork, I. Dos-Santos-Silva, A. M. Dunning, J. D. Eicher, P. A. Fasching, J. D. Faul, J. Figueroa, D. Flesch-Janys, I. Gandin, M. E. Garcia, M. Garcia-Closas, G. G. Giles, G. G. Girotto, M. S. Goldberg, A. Gonzalez-Neira, M. O. Goodarzi, M. L. Grove, D. F. Gudbjartsson, P. Guenel, X. Guo, C. A. Haiman, P. Hall, U. Hamann, B. E. Henderson, L. J. Hocking, A. Hofman, G. Homuth, M. J. Hooning, J. L. Hopper, F. B. Hu, J. Huang, K. Humphreys, D. J. Hunter, A. Jakubowska, S. E. Jones, M. Kabisch, D. Karasik, J. A. Knight, I. Kolcic, C. Kooperberg, V. M. Kosma, J. Kriebel, V. Kristensen, D. Lambrechts, C. Langenberg, J. Li, X. Li, S. Lindstrom, Y. Liu, J. Luan, J. Lubinski, R. Magi, A. Mannermaa, J. Manz, S. Margolin, J. Marten, N. G. Martin, C. Masciullo, A. Meindl, K. Michailidou, E. Mihailov, L. Milani, R. L. Milne, M. Muller-Nurasyid, M. Nalls, B. M. Neale, H. Nevanlinna, P. Neven, A. B. Newman, B. G. Nordestgaard, J. E. Olson, S. Padmanabhan, P. Peterlongo, U. Peters, A. Petersmann, J. Peto, P. D. P. Pharoah, N. N. Pirastu, A. Pirie, G. Pistis, O. Polasek, D. Porteous, B. M. Psaty, K. Pylkas, P. Radice, L. J. Raffel, F. Rivadeneira, I. Rudan, A. Rudolph, D. Ruggiero, C. F. Sala, S. Sanna, E. J. Sawyer, D. Schlessinger, M. K. Schmidt, F. Schmidt, R. K. Schmutzler, M. J. Schoemaker, R. A. Scott, C. M. Seynaeve, J. Simard, R. Sorice, M. C. Southey, D. Stockl, K. Strauch, A. Swerdlow, K. D. Taylor, U. Thorsteinsdottir, A. E. Toland, I. Tomlinson, T. Truong, L. Tryggvadottir, S. T. Turner, D. Vozzi, Q. Wang, M. Wellons, G. Willemsen, J. F. Wilson, R. Winqvist, Bbhr Wolffenbuttel, A. F. Wright, D. Yannoukakos, T. Zemunik, W. Zheng, M. Zygmunt, S. Bergmann, D. I. Boomsma, J. E. Buring, L. Ferrucci, G. W. Montgomery, V. Gudnason, T. D. Spector, C. M. van Duijn, B. Z. Alizadeh, M. Ciullo, L. Crisponi, D. F. Easton, P. P. Gasparini, C. Gieger, T. B. Harris, C. Hayward, S. L. R. Kardia, P. Kraft, B. McKnight, A. Metspalu, A. C. Morrison, A. P. Reiner, P. M. Ridker, J. I. Rotter, D. Toniolo, A. G. Uitterlinden, S. Ulivi, H. Volzke, N. J. Wareham, D. R. Weir, L. M. Yerges-Armstrong, Practical consortium, Investigators kConFab, Aocs Investigators, Scotland Generation, E. PIC-InterAct Consortium, Study LifeLines Cohort, A. L. Price, K. Stefansson, J. A. Visser, K. K. Ong, J. Chang-Claude, J. M. Murabito, J. R. B. Perry, and A. Murray. 2015. 'Large-scale genomic analyses link reproductive aging to hypothalamic signaling, breast cancer susceptibility and BRCA1-mediated DNA repair', *Nat Genet*, 47: 1294-303.

Day, F. R., D. J. Thompson, H. Helgason, D. I. Chasman, H. Finucane, P. Sulem, K. S. Ruth, S. Whalen, A. K. Sarkar, E. Albrecht, E. Altmaier, M. Amini, C. M. Barbieri, T. Boutin, A. Campbell, E. Demerath, A. Giri, C. He, J. J. Hottenga, R. Karlsson, I. Kolcic, P. R. Loh, K. L. Lunetta, M. Mangino, B. Marco, G. McMahon, S. E. Medland, I. M. Nolte, R. Noordam, T. Nutile, L. Paternoster, N. Perjakova, E. Porcu, L. M. Rose, K. E. Schraut, A. V. Segre, A. V. Smith, L. Stolk, A. Teumer, I. L. Andrulis, S. Bandinelli, M. W. Beckmann, J. Benitez, S. Bergmann, M. Bochud, E. Boerwinkle, S. E. Bojesen, M. K. Bolla, J. S.

Brand, H. Brauch, H. Brenner, L. Broer, T. Bruning, J. E. Buring, H. Campbell, E. Catamo, S. Chanock, G. Chenevix-Trench, T. Corre, F. J. Couch, D. L. Cousminer, A. Cox, L. Crisponi, K. Czene, G. Davey Smith, Ejcn de Geus, R. de Mutsert, I. De Vivo, J. Dennis, P. Devilee, I. Dos-Santos-Silva, A. M. Dunning, J. G. Eriksson, P. A. Fasching, L. Fernandez-Rhodes, L. Ferrucci, D. Flesch-Janys, L. Franke, M. Gabrielson, I. Gandin, G. G. Giles, H. Grallert, D. F. Gudbjartsson, P. Guenel, P. Hall, E. Hallberg, U. Hamann, T. B. Harris, C. A. Hartman, G. Heiss, M. J. Hooning, J. L. Hopper, F. Hu, D. J. Hunter, M. A. Ikram, H. K. Im, M. R. Jarvelin, P. K. Joshi, D. Karasik, M. Kellis, Z. Kutalik, G. LaChance, D. Lambrechts, C. Langenberg, L. J. Launer, J. S. E. Laven, S. Lenarduzzi, J. Li, P. A. Lind, S. Lindstrom, Y. Liu, J. Luan, R. Magi, A. Mannermaa, H. Mbarek, M. I. McCarthy, C. Meisinger, T. Meitinger, C. Menni, A. Metspalu, K. Michailidou, L. Milani, R. L. Milne, G. W. Montgomery, A. M. Mulligan, M. A. Nalls, P. Navarro, H. Nevanlinna, D. R. Nyholt, A. J. Oldehinkel, T. A. O'Mara, S. Padmanabhan, A. Palotie, N. Pedersen, A. Peters, J. Peto, P. D. P. Pharoah, A. Pouta, P. Radice, I. Rahman, S. M. Ring, A. Robino, F. R. Rosendaal, I. Rudan, R. Rueedi, D. Ruggiero, C. F. Sala, M. K. Schmidt, R. A. Scott, M. Shah, R. Sorice, M. C. Southey, U. Sovio, M. Stampfer, M. Steri, K. Strauch, T. Tanaka, E. Tikkanen, N. J. Timpson, M. Traglia, T. Truong, J. P. Tyrer, A. G. Uitterlinden, D. R. V. Edwards, V. Vitart, U. Volker, P. Vollenweider, Q. Wang, E. Widen, K. W. van Dijk, G. Willemsen, R. Winqvist, B. H. R. Wolffenbuttel, J. H. Zhao, M. Zoledziewska, M. Zygmunt, B. Z. Alizadeh, D. I. Boomsma, M. Ciullo, F. Cucca, T. Esko, N. Franceschini, C. Gieger, V. Gudnason, C. Hayward, P. Kraft, D. A. Lawlor, P. K. E. Magnusson, N. G. Martin, D. O. Mook-Kanamori, E. A. Nohr, O. Polasek, D. Porteous, A. L. Price, P. M. Ridker, H. Snieder, T. D. Spector, D. Stockl, D. Toniolo, S. Ulivi, J. A. Visser, H. Volzke, N. J. Wareham, J. F. Wilson, Study LifeLines Cohort, Consortium InterAct, Aocs Investigators kConFab, Consortium Endometrial Cancer Association, Consortium Ovarian Cancer Association, Practical consortium, A. B. Spurdle, U. Thorsteindottir, K. S. Pollard, D. F. Easton, J. Y. Tung, J. Chang-Claude, D. Hinds, A. Murray, J. M. Murabito, K. Stefansson, K. K. Ong, and J. R. B. Perry. 2017. 'Genomic analyses identify hundreds of variants associated with age at menarche and support a role for puberty timing in cancer risk', *Nat Genet*, 49: 834-41.

de Roux, N., E. Genin, J. C. Carel, F. Matsuda, J. L. Chaussain, and E. Milgrom. 2003. 'Hypogonadotropic hypogonadism due to loss of function of the KiSS1-derived peptide receptor GPR54', *Proc Natl Acad Sci U S A*, 100: 10972-6.

de Roux, N., J. Young, M. Misrahi, R. Genet, P. Chanson, G. Schaison, and E. Milgrom. 1997. 'A family with hypogonadotropic hypogonadism and mutations in the gonadotropin-releasing hormone receptor', *N Engl J Med*, 337: 1597-602.

Divall, S. A., T. R. Williams, S. E. Carver, L. Koch, J. C. Bruning, C. R. Kahn, F. Wondisford, S. Radovick, and A. Wolfe. 2010. 'Divergent roles of growth factors in the GnRH regulation of puberty in mice', *J Clin Invest*, 120: 2900-9.

Dode, C., and J. P. Hardelin. 2004. 'Kallmann syndrome: fibroblast growth factor signaling insufficiency?', *J Mol Med*, 82: 725-34.

Dode, C., J. Levilliers, J. M. Dupont, A. De Paepe, N. Le Du, N. Soussi-Yanicostas, R. S. Coimbra, S. Delmaghani, S. Compain-Nouaille, F. Baverel, C. Pecheux, D. Le Tessier, C. Cruaud, M. Delpech, F. Speleman, S. Vermeulen, A. Amalfitano, Y. Bachelot, P. Bouchard, S. Cabrol, J. C. Carel, H. Delemarre-Van De Waal, B. Goulet-Salmon, M. L. Kottler, O. Richard, F. Sanchez-Franco, R. Saura, J. Young, C. Petit, and J. P. Hardelin. 2003. 'Loss-of-function mutations in FGFR1 cause autosomal dominant Kallmann syndrome', *Nat Genet*, 33: 463-5.

Dode, C., and P. Rondard. 2013. 'PROK2/PROKR2 Signaling and Kallmann Syndrome', *Front Endocrinol (Lausanne)*, 4: 19.

Dode, C., L. Teixeira, J. Levilliers, C. Fouveaut, P. Bouchard, M. L. Kottler, J. Lespinasse, A. Lienhardt-Roussie, M. Mathieu, A. Moerman, G. Morgan, A. Murat, J. E. Toublanc, S. Wolczynski, M. Delpech, C. Petit, J. Young, and J. P. Hardelin. 2006. 'Kallmann syndrome: mutations in the genes encoding prokineticin-2 and prokineticin receptor-2', *PLoS Genet*, 2: e175.

Falardeau, J., W. C. Chung, A. Beenken, T. Raivio, L. Plummer, Y. Sidis, E. E. Jacobson-Dickman, A. V. Eliseenkova, J. Ma, A. Dwyer, R. Quinton, S. Na, J. E. Hall, C. Huot, N. Alois, S. H. Pearce, L. W.

Cole, V. Hughes, M. Mohammadi, P. Tsai, and N. Pitteloud. 2008. 'Decreased FGF8 signaling causes deficiency of gonadotropin-releasing hormone in humans and mice', *J Clin Invest*, 118: 2822-31.

Franco, B., S. Guioli, A. Pragliola, B. Incerti, B. Bardoni, R. Tonlorenzi, R. Carrozzo, E. Maestrini, M. Pieretti, P. Taillon-Miller, C.J. Brown, H.F. Willard, C. Lawrence, M. Persico, G. Camerino, and A. Ballabio. 1991. 'A gene deleted in Kallmann's syndrome shares homology with neural cell adhesion and axonal path-finding molecules', *Nature*, 353: 529-36.

Francou, B., C. Paul, L. Amazit, A. Cartes, C. Bouvattier, F. Albarel, D. Maiter, P. Chanson, S. Trabado, S. Brailly-Tabard, T. Brue, A. Guiochon-Mantel, J. Young, and J. Bouligand. 2016. 'Prevalence of KISS1 Receptor mutations in a series of 603 patients with normosmic congenital hypogonadotrophic hypogonadism and characterization of novel mutations: a single-centre study', *Hum Reprod*, 31: 1363-74.

Gajbhiye, R., J. N. Fung, and G. W. Montgomery. 2018. 'Complex genetics of female fertility', *NPJ Genom Med*, 3: 29.

Galazzi, E., P. Duminuco, M. Moro, F. Guizzardi, N. Marazzi, A. Sartorio, S. Avignone, M. Bonomi, L. Persani, and M. T. Bonati. 2018. 'Hypogonadotropic hypogonadism and pituitary hypoplasia as recurrent features in Ulnar-Mammary syndrome', *Endocr Connect*, 7: 1432-41.

Gianetti, E., C. Tusset, S. D. Noel, M. G. Au, A. A. Dwyer, V. A. Hughes, A. P. Abreu, J. Carroll, E. Trarbach, L. F. Silveira, E. M. Costa, B. B. de Mendonca, M. de Castro, A. Lofrano, J. E. Hall, E. Bolu, M. Ozata, R. Quinton, J. K. Amory, S. E. Stewart, W. Arlt, T. R. Cole, W. F. Crowley, U. B. Kaiser, A. C. Latronico, and S. B. Seminara. 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: 2857-67.

Goncalves, C. I., J. M. Aragues, M. Bastos, L. Barros, N. Vicente, D. Carvalho, and M. C. Lemos. 2017. 'GNRHR biallelic and digenic mutations in patients with normosmic congenital hypogonadotropic hypogonadism', *Endocr Connect*, 6: 360-66.

Habiby, R. L., P. Boepple, L. Nachtigall, P. M. Sluss, W. F. Crowley, Jr., and J. L. Jameson. 1996. 'Adrenal hypoplasia congenita with hypogonadotropic hypogonadism: evidence that DAX-1 mutations lead to combined hypothalmic and pituitary defects in gonadotropin production', *J Clin Invest*, 98: 1055-62.

Hanchate, N. K., P. Giacobini, P. Lhuillier, J. Parkash, C. Espy, C. Fouveaut, C. Leroy, S. Baron, C. Campagne, C. Vanacker, F. Collier, C. Cruaud, V. Meyer, A. Garcia-Pinero, D. Dewailly, C. Cortet-Rudelli, K. Gersak, C. Metz, G. Chabrier, M. Pugeat, J. Young, J. P. Hardelin, V. Prevot, and C. Dode. 2012. 'SEMA3A, a gene involved in axonal pathfinding, is mutated in patients with Kallmann syndrome', *PLoS Genet*, 8: e1002896.

Hardelin, J. P., J. Levilliers, S. Blanchard, J. C. Carel, M. Leutenegger, J. P. Pinard-Bertelletto, P. Bouloux, and C. Petit. 1993. 'Heterogeneity in the mutations responsible for X chromosome-linked Kallmann syndrome', *Hum Mol Genet*, 2: 373-7.

Howard, S. R., L. Guasti, G. Ruiz-Babot, A. Mancini, A. David, H. L. Storr, L. A. Metherell, M. J. Sternberg, C. P. Cabrera, H. R. Warren, M. R. Barnes, R. Quinton, N. de Roux, J. Young, A. Guiochon-Mantel, K. Wehkalampi, V. Andre, Y. Gothilf, A. Cariboni, and L. Dunkel. 2016. 'IGSF10 mutations dysregulate gonadotropin-releasing hormone neuronal migration resulting in delayed puberty', *EMBO Mol Med*, 8: 626-42.

Hutchins, B. I., L. D. Kotan, C. Taylor-Burds, Y. Ozkan, P. J. Cheng, F. Gurbuz, J. D. Tiong, E. Mengen, B. Yuksel, A. K. Topaloglu, and S. Wray. 2016. 'CCDC141 Mutation Identified in Anosmic Hypogonadotropic Hypogonadism (Kallmann Syndrome) Alters GnRH Neuronal Migration', *Endocrinology*, 157: 1956-66.

Jackson, R. S., J. W. Creemers, S. Ohagi, M. L. Raffin-Sanson, L. Sanders, C. T. Montague, J. C. Hutton, and S. O'Rahilly. 1997. 'Obesity and impaired prohormone processing associated with mutations in the human prohormone convertase 1 gene', *Nat Genet*, 16: 303-6.

Kansakoski, J., R. Fagerholm, E. M. Laitinen, K. Vaaralahti, P. Hackman, N. Pitteloud, T. Raivio, and J. Tommiska. 2014. 'Mutation screening of SEMA3A and SEMA7A in patients with congenital hypogonadotropic hypogonadism', *Pediatr Res*, 75: 641-4.

Kelberman, D., K. Rizzoti, A. Avilion, M. Bitner-Glindzicz, S. Cianfarani, J. Collins, W. K. Chong, J. M. Kirk, J. C. Achermann, R. Ross, D. Carmignac, R. Lovell-Badge, I. C. Robinson, and M. T. Dattani. 2006. 'Mutations within Sox2/SOX2 are associated with abnormalities in the hypothalamo-pituitary-gonadal axis in mice and humans', *J Clin Invest*, 116: 2442-55.

Kim, H. G., J. W. Ahn, I. Kurth, R. Ullmann, H. T. Kim, A. Kulharya, K. S. Ha, Y. Itokawa, I. Meliciani, W. Wenzel, D. Lee, G. Rosenberger, M. Ozata, D. P. Bick, R. J. Sherins, T. Nagase, M. Tekin, S. H. Kim, C. H. Kim, H. H. Ropers, J. F. Gusella, V. Kalscheuer, C. Y. Choi, and L. C. Layman. 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: 465-79.

Kim, H. G., S. R. Herrick, E. Lemyre, S. Kishikawa, J. A. Salisz, S. Seminara, M. E. MacDonald, G. A. Bruns, C. C. Morton, B. J. Quade, and J. F. Gusella. 2005. 'Hypogonadotropic hypogonadism and cleft lip and palate caused by a balanced translocation producing haploinsufficiency for FGFR1', *J Med Genet*, 42: 666-72.

Kim, H. G., I. Kurth, F. Lan, I. Meliciani, W. Wenzel, S. H. Eom, G. B. Kang, G. Rosenberger, M. Tekin, M. Ozata, D. P. Bick, R. J. Sherins, S. L. Walker, Y. Shi, J. F. Gusella, and L. C. Layman. 2008. 'Mutations in CHD7, encoding a chromatin-remodeling protein, cause idiopathic hypogonadotropic hypogonadism and Kallmann syndrome', *Am J Hum Genet*, 83: 511-9.

Kim, Y. J., D. P. Osborn, J. Y. Lee, M. Araki, K. Araki, T. Mohun, J. Kansakoski, N. Brandstack, H. T. Kim, F. Miralles, C. H. Kim, N. A. Brown, H. G. Kim, J. P. Martinez-Barbera, P. Ataliotis, T. Raivio, L. C. Layman, and S. H. Kim. 2018. 'WDR11-mediated Hedgehog signalling defects underlie a new ciliopathy related to Kallmann syndrome', *EMBO Rep*, 19: 269-89.

Kotan, L. D., C. Cooper, S. Darcan, I. M. Carr, S. Ozen, Y. Yan, M. K. Hamedani, F. Gurbuz, E. Mengen, I. Turan, A. Ulubay, G. Akkus, B. Yuksel, A. K. Topaloglu, and E. Leygue. 2016. 'Idiopathic Hypogonadotropic Hypogonadism Caused by Inactivating Mutations in SRA1', *J Clin Res Pediatr Endocrinol*, 8: 125-34.

Kotan, L. D., B. I. Hutchins, Y. Ozkan, F. Demirel, H. Stoner, P. J. Cheng, I. Esen, F. Gurbuz, Y. K. Bicakci, E. Mengen, B. Yuksel, S. Wray, and A. K. Topaloglu. 2014. 'Mutations in FEZF1 Cause Kallmann Syndrome', *Am J Hum Genet*, 95: 326-31.

Kotan, L. D., E. Isik, I. Turan, E. Mengen, G. Akkus, M. Tastan, F. Gurbuz, B. Yuksel, and A. K. Topaloglu. 2019. 'Prevalence and associated phenotypes of PLXNA1 variants in normosmic and anosmic idiopathic hypogonadotropic hypogonadism', *Clin Genet*, 95: 320-24.

Laitinen, E. M., J. Tommiska, T. Sane, K. Vaaralahti, J. Toppari, and T. Raivio. 2012. 'Reversible congenital hypogonadotropic hypogonadism in patients with CHD7, FGFR1 or GNRHR mutations', *PLoS One*, 7: e39450.

Laumonnier, F., N. Ronce, B. C. Hamel, P. Thomas, J. Lespinasse, M. Raynaud, C. Paringaux, H. Van Bokhoven, V. Kalscheuer, J. P. Fryns, J. Chelly, C. Moraine, and S. Briault. 2002. 'Transcription factor SOX3 is involved in X-linked mental retardation with growth hormone deficiency', *Am J Hum Genet*, 71: 1450-5.

Layman, L. C. 1999a. 'Genetics of human hypogonadotropic hypogonadism', *Am J Med Genet*, 89: 240-8.

———. 2002. 'Human gene mutations causing infertility', *J Med Genet*, 39: 153-61.

———. 2013a. 'Clinical genetic testing for kallmann syndrome', *J Clin Endocrinol Metab*, 98: 1860-2.

———. 2013b. 'The genetic basis of female reproductive disorders: Etiology and clinical testing', *Mol Cell Endocrinol*, 370: 138-48.

Layman, L. C., D. P. Cohen, M. Jin, J. Xie, Z. Li, R. H. Reindollar, S. Bolbolan, D. P. Bick, R. R. Sherins, L. W. Duck, L. C. Musgrove, J. C. Sellers, and J. D. Neill. 1998. 'Mutations in gonadotropin-releasing hormone receptor gene cause hypogonadotropic hypogonadism', *Nat Genet*, 18: 14-5.

Layman, L. C., E. J. Lee, D. B. Peak, A. B. Namnoum, K. V. Vu, B. L. van Lingen, M. R. Gray, P. G. McDonough, R. H. Reindollar, and J. L. Jameson. 1997. 'Delayed puberty and hypogonadism caused by mutations in the follicle-stimulating hormone beta-subunit gene', *N Engl J Med*, 337: 607-11.

Layman, L. C., A. L. Porto, J. Xie, L. A. da Motta, L. D. da Motta, W. Weiser, and P. M. Sluss. 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*, 87: 3702-7.

Layman, L.C. 1999b. 'Mutations in human gonadotropin genes and their physiologic significance in puberty and reproduction', *Fertil Steril*, 71: 201-18.

Legouis, R., J. P. Hardelin, J. Levilliers, J. M. Claverie, S. Compain, V. Wunderle, P. Millasseau, D. Le Paslier, D. Cohen, D. Caterina, L. Bougueleret, H. Delemarre-Van de Waal, G. Lutfalla, J. Weissenbach, and C. Petit. 1991. 'The candidate gene for the X-linked Kallmann syndrome encodes a protein related to adhesion molecules', *Cell*, 67: 423-35.

Lin, L., G. S. Conway, N. R. Hill, M. T. Dattani, P. C. Hindmarsh, and J. C. Achermann. 2006. 'A homozygous R262Q mutation in the gonadotropin-releasing hormone receptor presenting as constitutional delay of growth and puberty with subsequent borderline oligospermia', *J Clin Endocrinol Metab*, 91: 5117-21.

Lofrano-Porto, A., G. B. Barra, L. A. Giacomini, P. P. Nascimento, A. C. Latronico, L. A. Casulari, and A. da Rocha Neves Fde. 2007. 'Luteinizing hormone beta mutation and hypogonadism in men and women', *N Engl J Med*, 357: 897-904.

Machinis, K., J. Pantel, I. Netchine, J. Leger, O. J. Camand, M. L. Sobrier, F. Dastot-Le Moal, P. Duquesnoy, M. Abitbol, P. Czernichow, and S. Amselem. 2001. 'Syndromic short stature in patients with a germline mutation in the LIM homeobox LHX4', *Am J Hum Genet*, 69: 961-8.

Maione, L., S. Brailly-Tabard, J. Nevoux, J. Bouligand, and J. Young. 2016. 'Reversal of congenital hypogonadotropic hypogonadism in a man with Kallmann syndrome due to SOX10 mutation', *Clin Endocrinol (Oxf)*, 85: 988-89.

Maione, L., A. A. Dwyer, B. Francou, A. Guiochon-Mantel, N. Binart, J. Bouligand, and J. Young. 2018. 'GENETICS IN ENDOCRINOLOGY: Genetic counseling for congenital hypogonadotropic hypogonadism and Kallmann syndrome: new challenges in the era of oligogenism and next-generation sequencing', *Eur J Endocrinol*, 178: R55-R80.

Margolin, D. H., M. Kousi, Y. M. Chan, E. T. Lim, J. D. Schmahmann, M. Hadjivassiliou, J. E. Hall, I. Adam, A. Dwyer, L. Plummer, S. V. Aldrin, J. O'Rourke, A. Kirby, K. Lage, A. Milunsky, J. M. Milunsky, J. Chan, E. T. Hedley-Whyte, M. J. Daly, N. Katsanis, and S. B. Seminara. 2013. 'Ataxia, dementia, and hypogonadotropism caused by disordered ubiquitination', *N Engl J Med*, 368: 1992-2003.

Mason, A. J., J. S. Hayflick, R. T. Zoeller, W. S. Young, 3rd, H. S. Phillips, K. Nikolics, and P. H. Seeburg. 1986. 'A deletion truncating the gonadotropin-releasing hormone gene is responsible for hypogonadism in the hpg mouse', *Science*, 234: 1366-71.

Mason, A.J., S.L. Pitts, K. Nikolics, E. Szonyi, J.N. Wilcox, P.H. Seeburg, and T.A. Stewart. 1986. 'The hypogonadal mouse: reproductive functions restored by gene therapy.', *Science*, 234: 1372-78.

Matthews, C., and V.K. Chatterjee. 1997. 'Isolated deficiency of follicle-stimulating hormone re-revisited', *N Engl J Med*, 337: 642.

Matthews, C.H., S. Borgato, P. Beck-Peccoz, M. Adams, Y. Tone, G. Gambin, S. Casagrande, G. Tedeschini, A. Benedetti, and V.K.K. Chatterjee. 1993. 'Primary amenorrhea and infertility due to a mutation in the b-subunit of follicle-stimulating hormone', *Nat Genet*, 5: 83-86.

Maya-Nunez, G., S. Cuevas-Covarrubias, J. C. Zenteno, A. Ulloa-Aguirre, S. Kofman-Alfaro, and J. P. Mendez. 1998. 'Contiguous gene syndrome due to deletion of the first three exons of the Kallmann gene and complete deletion of the steroid sulphatase gene', *Clin Endocrinol (Oxf)*, 48: 713-8.

McCabe, M. J., C. Gaston-Massuet, V. Tziaferi, L. C. Gregory, K. S. Alatzoglou, M. Signore, E. Puelles, D. Gerrelli, I. S. Farooqi, J. Raza, J. Walker, S. I. Kavanaugh, P. S. Tsai, N. Pitteloud, J. P. Martinez-Barbera, and M. T. Dattani. 2011. 'Novel FGF8 mutations associated with recessive holoprosencephaly, craniofacial defects, and hypothalamo-pituitary dysfunction', *J Clin Endocrinol Metab*, 96: E1709-18.

McCormack, S. E., D. Li, Y. J. Kim, J. Y. Lee, S. H. Kim, R. Rapaport, and M. A. Levine. 2017. 'Digenic Inheritance of PROKR2 and WDR11 Mutations in Pituitary Stalk Interruption Syndrome', *J Clin Endocrinol Metab*, 102: 2501-07.

Messager, S., E. E. Chatzidaki, D. Ma, A. G. Hendrick, D. Zahn, J. Dixon, R. R. Thresher, I. Malinge, D. Lomet, M. B. Carlton, W. H. Colledge, A. Caraty, and S. A. Aparicio. 2005. 'Kisspeptin directly stimulates gonadotropin-releasing hormone release via G protein-coupled receptor 54', *Proc Natl Acad Sci U S A*, 102: 1761-6.

Miraoui, H., A. A. Dwyer, G. P. Sykiotis, L. Plummer, W. Chung, B. Feng, A. Beenken, J. Clarke, T. H. Pers, P. Dworzynski, K. Keefe, M. Niedziela, T. Raivio, W. F. Crowley, Jr., S. B. Seminara, R. Quinton, V.

A. Hughes, P. Kumanov, J. Young, M. A. Yialamas, J. E. Hall, G. Van Vliet, J. P. Chanoine, J. Rubenstein, M. Mohammadi, P. S. Tsai, Y. Sidis, K. Lage, and N. Pitteloud. 2013. 'Mutations in FGF17, IL17RD, DUSP6, SPRY4, and FLRT3 Are Identified in Individuals with Congenital Hypogonadotropic Hypogonadism', *Am J Hum Genet*, 92: 725-43.

Montague, C.T., S. Farooqi, F.P. Whitehead, M.A. Soos, H. Rau, N.J. Wareham, C.P. Sewter, J.E. Digby, S.N. Mohammed, J.A. Hurst, C.H. Cheetham, A.R. Earley, A.H. Barnett, J.B. Prins, and S. O'Rahilly. 1997. 'Congenital leptin deficiency is associated with severe early-onset obesity in humans', *Nature*, 387: 903-08.

Moya-Plana, A., C. Villanueva, O. Laccourreye, P. Bonfils, and N. de Roux. 2013. 'PROKR2 and PROK2 mutations cause isolated congenital anosmia without gonadotropic deficiency', *Eur J Endocrinol*, 168: 31-7.

Murcia-Belmonte, V., V. Astillero-Lopez, and P. F. Esteban. 2016. 'Anosmin 1 Interacts with the Prokineticin Receptor 2 In Vitro Indicating a Molecular Link Between Both Proteins in the Pathogenesis of Kallmann Syndrome', *Protein Pept Lett*, 23: 650-5.

Muscatelli, F., T. M. Strom, A. P. Walker, E. Zanaria, D. Recan, A. Meindl, B. Bardoni, S. Guioli, G. Zehetner, W. Rabl, and et al. 1994. 'Mutations in the DAX-1 gene give rise to both X-linked adrenal hypoplasia congenita and hypogonadotropic hypogonadism', *Nature*, 372: 672-6.

Netchine, I., M. L. Sobrier, H. Krude, D. Schnabel, M. Maghnie, E. Marcos, B. Duriez, V. Cacheux, A. Moers, M. Goossens, A. Gruters, and S. Amselem. 2000. 'Mutations in LHX3 result in a new syndrome revealed by combined pituitary hormone deficiency', *Nat Genet*, 25: 182-6.

Newbern, K., N. Natrajan, H. G. Kim, L. P. Chorich, L. M. Halvorson, R. S. Cameron, and L. C. Layman. 2013. 'Identification of HESX1 mutations in Kallmann syndrome', *Fertil Steril*, 99: 1831-7.

Pandurangi, M., M. Tamizharasi, and N. S. Reddy. 2015. 'Pregnancy outcome of assisted reproductive technology cycle in patients with hypogonadotropic hypogonadism', *J Hum Reprod Sci*, 8: 146-50.

Phillips, J. A., 3rd, J. S. Poling, C. A. Phillips, K. C. Stanton, E. D. Austin, J. D. Cogan, L. Wheeler, C. Yu, J. H. Newman, H. C. Dietz, and J. E. Loyd. 2008. 'Synergistic heterozygosity for TGFbeta1 SNPs and BMPR2 mutations modulates the age at diagnosis and penetrance of familial pulmonary arterial hypertension', *Genet Med*, 10: 359-65.

Pingault, V., V. Bodereau, V. Baral, S. Marcos, Y. Watanabe, A. Chaoui, C. Fouveaut, C. Leroy, O. Verier-Mine, C. Francannet, D. Dupin-Deguine, F. Archambeaud, F. J. Kurtz, J. Young, J. Bertherat, S. Marlin, M. Goossens, J. P. Hardelin, C. Dode, and N. Bondurand. 2013. 'Loss-of-Function Mutations in SOX10 Cause Kallmann Syndrome with Deafness', *Am J Hum Genet*, 92: 707-24.

Pitteloud, N., J. S. Acierno, Jr., A. Meysing, A. V. Eliseenkova, J. Ma, O. A. Ibrahimi, D. L. Metzger, F. J. Hayes, A. A. Dwyer, V. A. Hughes, M. Yialamas, J. E. Hall, E. Grant, M. Mohammadi, and W. F. Crowley, Jr. 2006. 'Mutations in fibroblast growth factor receptor 1 cause both Kallmann syndrome and normosmic idiopathic hypogonadotropic hypogonadism', *Proc Natl Acad Sci U S A*, 103: 6281-6.

Pitteloud, N., J. S. Acierno, Jr., A. U. Meysing, A. A. Dwyer, F. J. Hayes, and W. F. Crowley, Jr. 2005. 'Reversible kallmann syndrome, delayed puberty, and isolated anosmia occurring in a single family with a mutation in the fibroblast growth factor receptor 1 gene', *J Clin Endocrinol Metab*, 90: 1317-22.

Pitteloud, N., P. A. Boepple, S. DeCruz, S. B. Valkenburgh, W. F. Crowley, Jr., and F. J. Hayes. 2001. 'The fertile eunuch variant of idiopathic hypogonadotropic hypogonadism: spontaneous reversal associated with a homozygous mutation in the gonadotropin-releasing hormone receptor', *J Clin Endocrinol Metab*, 86: 2470-5.

Pitteloud, N., A. Meysing, R. Quinton, J. S. Acierno, Jr., A. A. Dwyer, L. Plummer, E. Fliers, P. Boepple, F. Hayes, S. Seminara, V. A. Hughes, J. Ma, P. Bouloux, M. Mohammadi, and W. F. Crowley, Jr. 2006. 'Mutations in fibroblast growth factor receptor 1 cause Kallmann syndrome with a wide spectrum of reproductive phenotypes', *Mol Cell Endocrinol*, 254-255: 60-9.

Pitteloud, N., R. Quinton, S. Pearce, T. Raivio, J. Acierno, A. Dwyer, L. Plummer, V. Hughes, S. Seminara, Y. Z. Cheng, W. P. Li, G. Maccoll, A. V. Eliseenkova, S. K. Olsen, O. A. Ibrahimi, F. J. Hayes, P. Boepple, J. E. Hall, P. Bouloux, M. Mohammadi, and W. Crowley. 2007. 'Digenic mutations account for variable phenotypes in idiopathic hypogonadotropic hypogonadism', *J Clin Invest*, 117: 457-63.

Pitteloud, N., C. Zhang, D. Pignatelli, J. D. Li, T. Raivio, L. W. Cole, L. Plummer, E. E. Jacobson-Dickman, P. L. Mellon, Q. Y. Zhou, and W. F. Crowley, Jr. 2007. 'Loss-of-function mutation in the prokineticin 2 gene causes Kallmann syndrome and normosmic idiopathic hypogonadotropic hypogonadism', *Proc Natl Acad Sci U S A*, 104: 17447-52.

Quaynor, S. D., M. E. Bosley, C. G. Duckworth, K. R. Porter, S. H. Kim, H. G. Kim, L. P. Chorich, M. E. Sullivan, J. H. Choi, R. S. Cameron, and L. C. Layman. 2016. 'Targeted next generation sequencing approach identifies eighteen new candidate genes in normosmic hypogonadotropic hypogonadism and Kallmann syndrome', *Mol Cell Endocrinol*, 437: 86-96.

Quaynor, S. D., H. G. Kim, E. M. Cappello, T. Williams, L. P. Chorich, D. P. Bick, R. J. Sherins, and L. C. Layman. 2011. 'The prevalence of digenic mutations in patients with normosmic hypogonadotropic hypogonadism and Kallmann syndrome', *Fertil Steril*, 96: 1424-30 e6.

Quaynor, S. D., E. K. Ko, L. P. Chorich, M. E. Sullivan, D. Demir, J. L. Waller, H. G. Kim, R. S. Cameron, and L. C. Layman. 2015. 'NELF knockout is associated with impaired pubertal development and subfertility', *Mol Cell Endocrinol*, 407: 26-36.

Quinton, R., V. M. Duke, A. Robertson, J. M. Kirk, G. Matfin, P. A. de Zoysa, C. Azcona, G. S. MacColl, H. S. Jacobs, G. S. Conway, M. Besser, R. G. Stanhope, and P. M. Bouloux. 2001. 'Idiopathic gonadotrophin deficiency: genetic questions addressed through phenotypic characterization', *Clin Endocrinol (Oxf)*, 55: 163-74.

Raivio, T., J. Falardeau, A. Dwyer, R. Quinton, F. J. Hayes, V. A. Hughes, L. W. Cole, S. H. Pearce, H. Lee, P. Boepple, W. F. Crowley, Jr., and N. Pitteloud. 2007. 'Reversal of Idiopathic Hypogonadotropic Hypogonadism', *N Engl J Med*, 357: 863-73.

Raivio, T., Y. Sidis, L. Plummer, H. Chen, J. Ma, A. Mukherjee, E. Jacobson-Dickman, R. Quinton, G. Van Vliet, H. Lavoie, V. A. Hughes, A. Dwyer, F. J. Hayes, S. Xu, S. Sparks, U. B. Kaiser, M. Mohammadi, and N. Pitteloud. 2009. 'Impaired fibroblast growth factor receptor 1 signaling as a cause of normosmic idiopathic hypogonadotropic hypogonadism', *J Clin Endocrinol Metab*, 94: 4380-90.

Rastrelli, G., G. Corona, E. Mannucci, and M. Maggi. 2014. 'Factors affecting spermatogenesis upon gonadotropin-replacement therapy: a meta-analytic study', *Andrology*, 2: 794-808.

Ribeiro, R. S., T. C. Vieira, and J. Abucham. 2007. 'Reversible Kallmann syndrome: report of the first case with a KAL1 mutation and literature review', *Eur J Endocrinol*, 156: 285-90.

Richards, S., N. Aziz, S. Bale, D. Bick, S. Das, J. Gastier-Foster, W. W. Grody, M. Hegde, E. Lyon, E. Spector, K. Voelkerding, H. L. Rehm, and Acmg Laboratory Quality Assurance Committee. 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: 405-24.

Rugarli, E. I., and A. Ballabio. 1993. 'Kallmann syndrome. From genetics to neurobiology', *JAMA*, 270: 2713-6.

Salenave, S., P. Chanson, H. Bry, M. Pugeat, S. Cabrol, J. C. Carel, A. Murat, P. Lecomte, S. Brailly, J. P. Hardelin, C. Dode, and J. Young. 2008. 'Kallmann's syndrome: a comparison of the reproductive phenotypes in men carrying KAL1 and FGFR1/KAL2 mutations', *J Clin Endocrinol Metab*, 93: 758-63.

Salian-Mehta, S., M. Xu, A. J. Knox, L. Plummer, D. Slavov, M. Taylor, S. Bevers, R. S. Hodges, W. F. Crowley, Jr., and M. E. Wierman. 2014. 'Functional consequences of AXL sequence variants in hypogonadotropic hypogonadism', *J Clin Endocrinol Metab*, 99: 1452-60.

Santoro, N., M. Filicori, and W. F. Crowley, Jr. 1986. 'Hypogonadotropic disorders in men and women: diagnosis and therapy with pulsatile gonadotropin-releasing hormone', *Endocr Rev*, 7: 11-23.

Sarfati, J., A. Guiochon-Mantel, P. Rondard, I. Arnulf, A. Garcia-Pinero, S. Wolczynski, S. Brailly-Tabard, M. Bidet, M. Ramos-Arroyo, M. Mathieu, A. Lienhardt-Roussie, G. Morgan, Z. Turki, C. Bremont, J. Lespinasse, H. Du Boullay, N. Chabbert-Buffet, S. Jacquemont, G. Reach, N. De Talence, P. Tonella, B. Conrad, F. Despert, B. Delobel, T. Brue, C. Bouvattier, S. Cabrol, M. Pugeat, A. Murat, P. Bouchard, J. P. Hardelin, C. Dode, and J. Young. 2010. 'A comparative phenotypic study of kallmann syndrome patients carrying monoallelic and biallelic mutations in the prokineticin 2 or prokineticin receptor 2 genes', *J Clin Endocrinol Metab*, 95: 659-69.

Sato, N., K. Ohyama, M. Fukami, M. Okada, and T. Ogata. 2006. 'Kallmann syndrome: somatic and germline mutations of the fibroblast growth factor receptor 1 gene in a mother and the son', *J Clin Endocrinol Metab*, 91: 1415-8.

Schaffer, A. A. 2013. 'Digenic inheritance in medical genetics', *J Med Genet*, 50: 641-52.

Schuler, A. M., B. A. Gower, D. Matern, P. Rinaldo, J. Vockley, and P. A. Wood. 2005. 'Synergistic heterozygosity in mice with inherited enzyme deficiencies of mitochondrial fatty acid beta-oxidation', *Mol Genet Metab*, 85: 7-11.

Seminara, S. B. 2007. 'Kisspeptin in reproduction', *Semin Reprod Med*, 25: 337-43.

Seminara, S. B., M. Beranova, L. M. Oliveira, K. A. Martin, W. F. Crowley, Jr., and J. E. Hall. 2000. 'Successful use of pulsatile gonadotropin-releasing hormone (GnRH) for ovulation induction and pregnancy in a patient with GnRH receptor mutations', *J Clin Endocrinol Metab*, 85: 556-62.

Seminara, S. B., S. Messager, E. E. Chatzidaki, R. R. Thresher, J. S. Acierno, Jr., J. K. Shagoury, Y. Bo-Abbas, W. Kuohung, K. M. Schwinof, A. G. Hendrick, D. Zahn, J. Dixon, U. B. Kaiser, S. A. Slaugenhaupt, J. F. Gusella, S. O'Rahilly, M. B. Carlton, W. F. Crowley, Jr., S. A. Aparicio, and W. H. Colledge. 2003. 'The GPR54 gene as a regulator of puberty', *N Engl J Med*, 349: 1614-27.

Shaw, N. D., H. Brand, Z. A. Kupchinsky, H. Bengani, L. Plummer, T. I. Jones, S. Erdin, K. A. Williamson, J. Rainger, A. Stortchevoi, K. Samocha, B. B. Currall, D. S. Dunican, R. L. Collins, J. R. Willer, A. Lek, M. Lek, M. Nassan, S. Pereira, T. Kammin, D. Lucente, A. Silva, C. M. Seabra, C. Chiang, Y. An, M. Ansari, J. K. Rainger, S. Joss, J. C. Smith, M. F. Lippincott, S. S. Singh, N. Patel, J. W. Jing, J. R. Law, N. Ferraro, A. Verloes, A. Rauch, K. Steindl, M. Zweier, I. Scheer, D. Sato, N. Okamoto, C. Jacobsen, J. Tryggestad, S. Chernausek, L. A. Schimmenti, B. Brasseur, C. Cesaretti, J. E. Garcia-Ortiz, T. P. Buitrago, O. P. Silva, J. D. Hoffman, W. Muhlbauer, K. W. Ruprecht, B. L. Loeys, M. Shino, A. M. Kaindl, C. H. Cho, C. C. Morton, R. R. Meehan, V. van Heyningen, E. C. Liao, R. Balasubramanian, J. E. Hall, S. B. Seminara, D. Macarthur, S. A. Moore, K. I. Yoshiura, J. F. Gusella, J. A. Marsh, J. M. Graham, Jr., A. E. Lin, N. Katsanis, P. L. Jones, W. F. Crowley, Jr., E. E. Davis, D. R. FitzPatrick, and M. E. Talkowski. 2017. 'SMCHD1 mutations associated with a rare muscular dystrophy can also cause isolated arhinia and Bosma arhinia microphthalmia syndrome', *Nat Genet*, 49: 238-48.

Shaw, N. D., S. B. Seminara, C. K. Welt, M. G. Au, L. Plummer, V. A. Hughes, A. A. Dwyer, K. A. Martin, R. Quinton, V. Mericq, P. M. Merino, J. F. Gusella, W. F. Crowley, Jr., N. Pitteloud, and J. E. Hall. 2011. 'Expanding the phenotype and genotype of female GnRH deficiency', *J Clin Endocrinol Metab*, 96: E566-76.

Shi, C. H., J. C. Schisler, C. E. Rubel, S. Tan, B. Song, H. McDonough, L. Xu, A. L. Portbury, C. Y. Mao, C. True, R. H. Wang, Q. Z. Wang, S. L. Sun, S. B. Seminara, C. Patterson, and Y. M. Xu. 2014. 'Ataxia and hypogonadism caused by the loss of ubiquitin ligase activity of the U box protein CHIP', *Hum Mol Genet*, 23: 1013-24.

Sidhoum, V. F., Y. M. Chan, M. F. Lippincott, R. Balasubramanian, R. Quinton, L. Plummer, A. Dwyer, N. Pitteloud, F. J. Hayes, J. E. Hall, K. A. Martin, P. A. Boepple, and S. B. Seminara. 2014. 'Reversal and relapse of hypogonadotropic hypogonadism: resilience and fragility of the reproductive neuroendocrine system', *J Clin Endocrinol Metab*, 99: 861-70.

Simonis, N., I. Migeotte, N. Lambert, C. Perazzolo, D. C. de Silva, B. Dimitrov, C. Heinrichs, S. Janssens, B. Kerr, G. Mortier, G. Van Vliet, P. Lepage, G. Casimir, M. Abramowicz, G. Smits, and C. Vilain. 2013. 'FGFR1 mutations cause Hartsfield syndrome, the unique association of holoprosencephaly and ectrodactyly', *J Med Genet*, 50: 585-92.

Sinisi, A. A., R. Asci, G. Bellastella, L. Maione, D. Esposito, A. Elefante, A. De Bellis, A. Bellastella, and A. Iolascon. 2008. 'Homozygous mutation in the prokineticin-receptor2 gene (Val274Asp) presenting as reversible Kallmann syndrome and persistent oligozoospermia: case report', *Hum Reprod*, 23: 2380-4.

Spilker, C., S. Nullmeier, K. M. Grochowska, A. Schumacher, I. Butnaru, T. Macharadze, G. M. Gomes, P. Yuanxiang, G. Bayraktar, C. Rodenstein, C. Geiseler, A. Kolodziej, J. Lopez-Rojas, D. Montag, F. Angenstein, J. Bar, W. D'Hanis, T. Roskoden, M. Mikhaylova, E. Budinger, F. W. Ohl, O. Stork, A. C. Zenclussen, A. Karpova, H. Schwegler, and M. R. Kreutz. 2016. 'A Jacob/Nsmf Gene Knockout Results in Hippocampal Dysplasia and Impaired BDNF Signaling in Dendritogenesis', *PLoS Genet*, 12: e1005907.

Strobel, A., T. Issad, L. Camoin, M. Ozata, and A.D. Strosberg. 1998. 'A leptin missense mutation associated with hypogonadism and morbid obesity', *Nat Genet*, 18: 213-15.

Suwinski, P., C. Ong, M. H. T. Ling, Y. M. Poh, A. M. Khan, and H. S. Ong. 2019. 'Advancing Personalized Medicine Through the Application of Whole Exome Sequencing and Big Data Analytics', *Front Genet*, 10: 49.

Sykiotis, G. P., L. Plummer, V. A. Hughes, M. Au, S. Durrani, S. Nayak-Young, A. A. Dwyer, R. Quinton, J. E. Hall, J. F. Gusella, S. B. Seminara, W. F. Crowley, Jr., and N. Pitteloud. 2010. 'Oligogenic basis of isolated gonadotropin-releasing hormone deficiency', *Proc Natl Acad Sci U S A*, 107: 15140-4.

Tajima, T., T. Hattori, T. Nakajima, K. Okuhara, J. Tsubaki, and K. Fujieda. 2007. 'A novel missense mutation (P366T) of the LHX4 gene causes severe combined pituitary hormone deficiency with pituitary hypoplasia, ectopic posterior lobe and a poorly developed sella turcica', *Endocr J*, 54: 637-41.

Tata, B., L. Huijbregts, S. Jacquier, Z. Csaba, E. Genin, V. Meyer, S. Leka, J. Dupont, P. Charles, D. Chevenne, J. C. Carel, J. Leger, and N. de Roux. 2014. 'Haploinsufficiency of Dmxl2, encoding a synaptic protein, causes infertility associated with a loss of GnRH neurons in mouse', *PLoS Biol*, 12: e1001952.

Topaloglu, A. K., A. Lomniczi, D. Kretzschmar, G. A. Dissen, L. D. Kotan, C. A. McArdle, A. F. Koc, B. C. Hamel, M. Guclu, E. D. Papatya, E. Eren, E. Mengen, F. Gurbuz, M. Cook, J. M. Castellano, M. B. Kekil, N. O. Mungan, B. Yuksel, and S. R. Ojeda. 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: E2067-75.

Topaloglu, A. K., F. Reimann, M. Guclu, A. S. Yalin, L. D. Kotan, K. M. Porter, A. Serin, N. O. Mungan, J. R. Cook, M. N. Ozbek, S. Imamoglu, N. S. Akalin, B. Yuksel, S. O'Rahilly, and R. K. Semple. 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: 354-8.

Topaloglu, A. K., J. A. Tello, L. D. Kotan, M. N. Ozbek, M. B. Yilmaz, S. Erdogan, F. Gurbuz, F. Temiz, R. P. Millar, and B. Yuksel. 2012. 'Inactivating KISS1 mutation and hypogonadotropic hypogonadism', *N Engl J Med*, 366: 629-35.

Tornberg, J., G. P. Sykiotis, K. Keefe, L. Plummer, X. Hoang, J. E. Hall, R. Quinton, S. B. Seminara, V. Hughes, G. Van Vliet, S. Van Uum, W. F. Crowley, H. Habuchi, K. Kimata, N. Pitteloud, and H. E. Bulow. 2011. 'Heparan sulfate 6-O-sulfotransferase 1, a gene involved in extracellular sugar modifications, is mutated in patients with idiopathic hypogonadotrophic hypogonadism', *Proc Natl Acad Sci U S A*, 108: 11524-9.

Trofimova, T., D. Lizneva, L. Suturina, W. Walker, Y. H. Chen, R. Azziz, and L. C. Layman. 2017. 'Genetic basis of eugonadal and hypogonadal female reproductive disorders', *Best Pract Res Clin Obstet Gynaecol*, 44: 3-14.

Valdes-Socin, H., R. Salvi, A. F. Daly, R. C. Gaillard, P. Quatresooz, P. M. Tebeu, F. P. Pralong, and A. Beckers. 2004. 'Hypogonadism in a patient with a mutation in the luteinizing hormone beta-subunit gene', *N Engl J Med*, 351: 2619-25.

Vissers, L. E., C. M. van Ravenswaaij, R. Admiraal, J. A. Hurst, B. B. de Vries, I. M. Janssen, W. A. van der Vliet, E. H. Huys, P. J. de Jong, B. C. Hamel, E. F. Schoenmakers, H. G. Brunner, J. A. Veltman, and A. G. van Kessel. 2004. 'Mutations in a new member of the chromodomain gene family cause CHARGE syndrome', *Nat Genet*, 36: 955-7.

Vockley, J., P. Rinaldo, M. J. Bennett, D. Matern, and G. D. Vladutiu. 2000. 'Synergistic heterozygosity: disease resulting from multiple partial defects in one or more metabolic pathways', *Mol Genet Metab*, 71: 10-8.

Weiss, J., L. Axelrod, R.W. Whitcomb, P.E. Harris, W.F. Crowley, Jr., and J.L. Jameson. 1992. 'Hypogonadism caused by a single amino acid substituion in the b subunit of luteinizing hormone', *N Engl J Med*, 326: 179-83.

Wu, W., J.D. Cogan, R.W. Pfaffle, J.S. Dasen, H. Frisch, S.M. O'Connell, S.E. Flynn, M.R. Brown, P.E. Mullis, J.S. Parks, J.A. Phillips III, and M.G. Rosenfeld. 1998. 'Mutations in PROP1 cause familial combined pituitary hormone deficiency', *Nat Genet*, 18: 147-49.

Xu, C., A. Messina, E. Somm, H. Miraoui, T. Kinnunen, J. Acierno, Jr., N. J. Niederlander, J. Bouilly, A. A. Dwyer, Y. Sidis, D. Cassatella, G. P. Sykiotis, R. Quinton, C. De Geyter, M. Dirlewanger, V.

Schwitzgebel, T. R. Cole, A. A. Toogood, J. M. Kirk, L. Plummer, U. Albrecht, W. F. Crowley, Jr., M. Mohammadi, M. Tena-Sempere, V. Prevot, and N. Pitteloud. 2017. 'KLB, encoding beta-Klotho, is mutated in patients with congenital hypogonadotropic hypogonadism', *EMBO Mol Med*, 9: 1379-97.

Xu, N., H. G. Kim, B. Bhagavath, S. G. Cho, J. H. Lee, K. Ha, I. Meliciani, W. Wenzel, R. H. Podolsky, L. P. Chorich, K. A. Stackhouse, A. M. Grove, L. N. Odom, M. Ozata, D. P. Bick, R. J. Sherins, S. H. Kim, R. S. Cameron, and L. C. Layman. 2011. 'Nasal embryonic LHRH factor (NELF) mutations in patients with normosmic hypogonadotropic hypogonadism and Kallmann syndrome', *Fertil Steril*, 95: 1613-20 e1-7.

Young, J., C. Metay, J. Bouligand, B. Tou, B. Francou, L. Maione, L. Tosca, J. Sarfati, F. Brioude, B. Esteva, A. Briand-Suleau, S. Brisset, M. Goossens, G. Tachdjian, and A. Guiochon-Mantel. 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: 1460-5.

Yu, R.N., M. Ito, T.L. Saunders, S.A. Camper, and J.L. Jameson. 1998. 'Role of Ahch in gonadal development and gametogenesis', *Nat Genet*, 20: 353-57.

Zanaria, E., F. Muscatelli, B. Bardoni, T. M. Strom, S. Guioli, W. Guo, E. Lalli, C. Moser, A. P. Walker, E. R. McCabe, T. Meitinger, A.P. Monace, P. Sassone-Corsi, and G. Camerino. 1994. 'An unusual member of the nuclear hormone receptor superfamily responsible for X-linked adrenal hypoplasia congenita', *Nature*, 372: 635-41.

Zhou, C., Y. Niu, H. Xu, Z. Li, T. Wang, W. Yang, S. Wang, D. W. Wang, and J. Liu. 2018. 'Mutation profiles and clinical characteristics of Chinese males with isolated hypogonadotropic hypogonadism', *Fertil Steril*, 110: 486-95 e5.

Zhu, Z., X. Han, Y. Li, C. Han, M. Deng, Y. Zhang, Q. Shen, Y. Cao, Z. Li, X. Wang, J. Gu, X. Liu, Y. Yang, Q. Zhang, and F. Hu. 2020. 'Identification of ROBO1/2 and SCEL as candidate genes in Kallmann syndrome with emerging bioinformatic analysis', *Endocrine*, 67: 224-32.

Highlights

-Of the 44 human normosmic hypogonadotropic hypogonadism (nHH)/Kallmann syndrome (KS) genes, >90% are autosomal in inheritance
-With digenic occurrence, most variants occur in genes associated with both nHH and KS that are inherited in an autosomal dominant fashion
-Variants in FGFR1 constitute the most common cause of monogenic and digenic nHH/KS
-Of the 43 homozygous mouse models, nearly half were lethal, 20% displayed hypogonadotropic hypogonadism, and most of the rest had subfertility
-Genetic testing may be accomplished by targeted gene sequencing based upon sense of smell, associated anomalies, inheritance patterns, or by next generation sequencing/Sanger confirmation with reflex deletion testing

Figure Legends

Figure 1: Chromosome location of nHH/KS genes beginning with chromosome 1 and ending with chromosome X. Chro = chromosome. Shown are the 44 genes in OMIM as well as the 14 genes of uncertain significance.

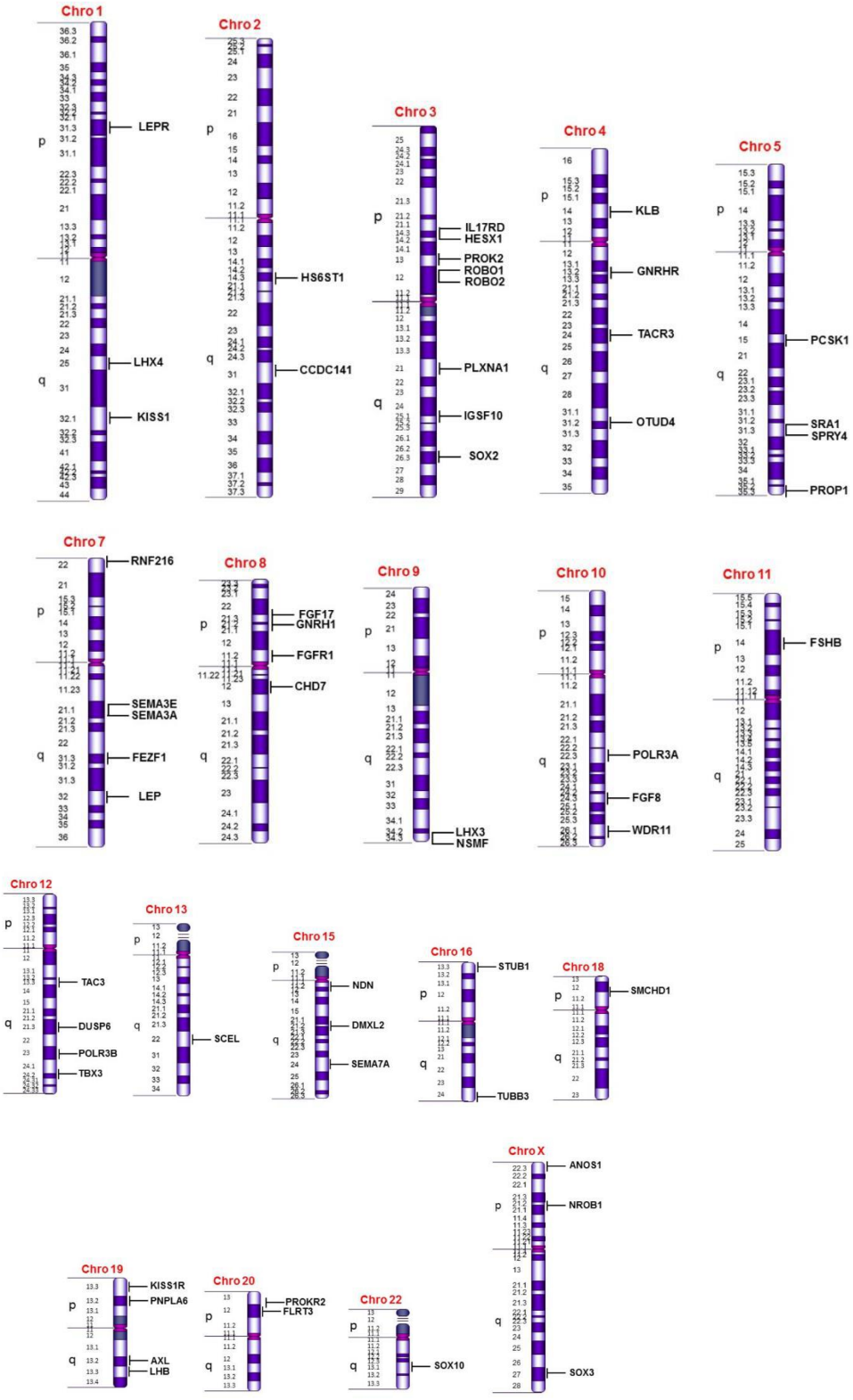
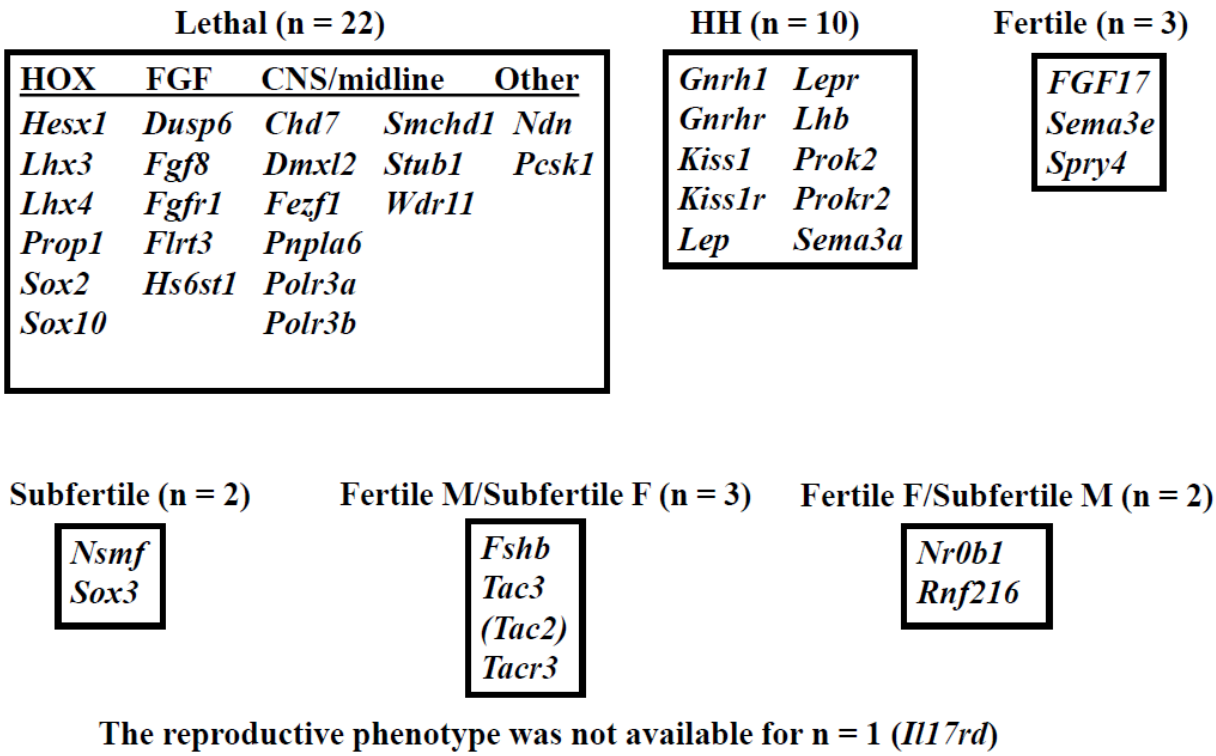


Figure 2: Mouse models of the homozygous loss of function genes in broad categories. HH = hypogonadotropic hypogonadism; M = male; F = female. It is recognized that other phenotypic features are present in addition to those shown here, but they were selected in these major categories.



The reproductive phenotype was not available for n = 1 (*Il17rd*)

Figure 3. Digenic cases where both variants were confirmed in vitro. These correspond to Cases 1-8 in Table 5. Cases 1 and 2 are from Pitteloud et al. J Clin Invest (2007); cases 3 and 4 from Ravio et al. JCEM 2008. Cases 5, 6, and 7 are from Hanchate et al PLOS Genet 2012, but pedigrees were not provided. Case 8 is from McCormack et al. JCEM 2017. CLD = clinodactyly; DS = Duane syndrome; MLD = midline facial defect; CPHD = combined pituitary hormone deficiency.