THE UNIVERSITY OF CHICAGO | Genetic Services Laboratories

Next Generation Sequencing Panel for Hypogonadotropic Hypogonadism

Clinical Features:

Male hypogonadism is characterized by impaired testicular function, which can affect spermatogenesis and/or testosterone synthesis. Male hypogonadism that occur secondary to hypothalamic-pituitary dysfunction is known as hypogonadotropic hypogonadism (HH). In HH, secretion of gonadotropin releasing hormone (GnRh) is absent or inadequate [1]. HH can be congenital or acquired. Congenital HH is divided in two main clinical phenotypes depending on the presence of an intact sense of smell: anosmic HH (Kallman syndrome) and congenital normosmic isolated hypogonadotropic hypogonadism (idiopathic HH or IHH). Approximately 60% of individuals with IHH have a defective sense of smell. Patients with Kallman syndrome (KS) may have additional phenotypic abnormalities including craniofacial defects, neurosensory deafness, digital anomalies, unilateral renal agenesis, and neurological defects, whereas normosmic IHH is usually not associated with any other malformations [2]. Congenital IHH is a clinically and genetically heterogeneous disorder. Although sporadic cases are the most frequent, families with congenital IHH have been reported with X-linked, autosomal dominant or recessive inheritance. A growing list of genes has been implicated in the molecular pathogenesis of congenital IHH with the molecular basis of this condition being identified in ~ 30% of patient. These genes encode neuropeptides and protein involved in the development and migration of GnRH neurons, or in the control of different stages of GnRH function. Management of IHH may include administration of gonadal steroids to stimulate development of secondary sex characteristics followed by infertility treatment in adulthood [3]. Early intervention can prevent low bone density and related complications, and also provides the opportunity for early family planning.

Our Hypogonadotropic Hypogonadism Sequencing Panel includes the 19 genes listed below.
*Our Kallmann Syndrome Panel includes the 16 genes listed in **bold** below*

Hypogonadotropic Hypogonadism Sequencing Panel			
CHD7	*FSHB*	***KISS1R***	***PROKR2***
FEZF1	***GNRH1***	***LHB***	***TAC3***
FGF17	*GNRHR*	*NR0B1 (DAX1)*	***TACR3***
FGF8	***IL17RD***	***NSMF (NELF)***	***WDR11***
FGFR1 (KAL2)	***KAL1***	***PROK2***	

Gene	Clinical Features	Details
CHD7 [OMIM# 608892]	CHARGE, KS and IHH	Kim *et al.* (2008) analyzed the *CHD7* gene in 197 patients with KS or normosmic IHH and identified 7 different heterozygous mutations in 7 sporadic patients, 3 with KS and 4 with IHH, respectively [4]. The authors concluded that both normosmic IHH and Kallmann syndrome due to *CHD7* mutations are mild allelic variants of CHARGE syndrome.
FEZF1 [OMIM#613301]	KS	Kotan *et al.* (2014) performed whole-exome sequencing in a cohort of 30 individuals with hypogonadotropic hypogonadism and anosmia, in whom mutations in known KS-associated genes had been excluded. In 4 affected individuals from 2 unrelated consanguineous Kurdish families, they identified homozygosity for a missense mutation and a 1-bp deletion respectively, in the *FEZF1* gene [5].
FGF17 [OMIM603725]	KS and IHH	In a cohort of 386 unrelated individuals with congenital HH, 199 of whom were anosmic and 187 normosmic, Miraoui *et al.* (2013) identified 3 HH probands with heterozygous missense mutations in the *FGF17* gene. Of the 3 probands with *FGF17* mutations, 2 were anosmic and 1 was normosmic; additional features included low bone mass in 2 of the patients [6].
FGF8 [OMIM# 600483]	KS and IHH	Falardeau *et al.* (2008) screened the *FGF8* gene in 461 unrelated probands with IHH, including 193 normosmic patients, 237 anosmic patients, and 21 patients with adult-onset IHH and identified 6 mutations

		in the *FGF8* gene. Structural and *in vitro* biochemical analysis of the *FGF8* mutations demonstrated that the 6 mutations resulted in loss of function.
FGFR1 (KAL2) [OMIM#136350]	KS and IHH	Heterozygous mutations in the *FGFR1* gene have been identified in several patients with KS and IHH, including missense and splice site mutations [8, 9]. Notably, as many as 30% of the *FGFR1* mutations found in the patients could be *de novo* mutations.
FSHB [OMIM#136530]	IHH	In an Italian woman with primary amenorrhea and infertility associated with isolated deficiency of pituitary FSH and normal luteinizing hormone secretion, Matthews *et al.* (1993) [10] identified homozygosity for a 2-bp frameshift deletion in the *FSHB* gene. Biallelic mutations in *FSHB* have also been identified in men with hypogonadism and isolated deficiency of FSH [11] as well as in men with infertility, azoospermia, and undetectable FSH [12].
GNRH1 [OMIM#152760]	KS and IHH	In an 18-year-old Romanian man who had normosmic hypogonadotropic hypogonadism, Bouligand *et al.* (2009) identified homozygosity for a 1-bp insertion in the *GNRH1* gene. His affected sister was also homozygous for the mutation, and his unaffected parents and an unaffected sister were heterozygotes, as was 1 of 200 ancestrally matched Romanian controls [13]. This mutation results in an aberrant peptide lacking the conserved GnRH decapeptide sequence, as shown by the absence of immunoreactive GnRH when expressed in vitro.
GNRHR [OMIM#138850]	IHH	Homozygous or compound heterozygous mutations in the *GNRHR* gene have been identified in a number of patients with IHH [14, 15].
IL17RD [OMIM#606807]	KS	In a cohort of 386 unrelated individuals with congenital HH, 199 of whom were anosmic and 187 normosmic, Miraoui *et al.* (2013) identified 6 HH probands who were heterozygous and 2 who were homozygous for missense mutations in the *IL17RD* gene [6]. Two of the patients with heterozygous *IL17RD* mutations also carried a heterozygous missense mutation in another HH-associated gene, *FGFR1* and *KISS1R* respectively. All of the patients were anosmic; 7 had absent puberty and 1 had partial puberty.
KAL1 [OMIM#300836]	KS	Missense and truncating mutations in the X-linked gene *KAL1* account for roughly 8% of all KS cases [16]. Mutations in *KAL1* are mainly nonsense mutations, frameshift mutations, or large gene deletions. Female carriers of *KAL1* mutations are typically clinically unaffected.
KISS1R [OMIM#603286; 604161]	IHH and CPP	Loss-of-function mutations in the *KISS1* genes are associated with IHH, while gain-of-function mutations have been associated with central precious puberty (CPP), which leads to premature development of secondary sexual characteristics, acceleration in linear growth, and bone age advancement [17].
LHB [OMIM#152780]	IHH	Homozygous or compound heterozygous mutation in the *LHB* gene cause IHH due to isolated luteinizing hormone (LH) deficiency. Male patients have normal sexual differentiation but fail to develop spontaneous puberty. Absence of LH alters Leydig cell proliferation and maturation and impairs the onset of normal spermatogenesis, which requires high levels of intratesticular testosterone. Female patients exhibit normal pubertal development and menarche, followed by oligomenorrhea and anovulatory secondary amenorrhea[18].
NR0B1 (DAX1) [OMIM#300473]	ACH, IHH	In males, mutations in the X-linked *NR0B1* gene may cause HH secondary to adrenal failure due to congenital adrenal hypoplasia (AHC). Most female carriers of *NR0B1* mutations have normal adrenal function and no evidence of HH [19].
NSMF (NELF) [OMIM#608137]	KS and IHH	Heterozygous mutations in the *NELF* gene (*NSMF*) cause HH with or without anosmia [20].
PROK2 and *PROKR2* [OMIM#607002; 607123]	KS and IHH	In a study of 192 patients with Kallmann syndrome, Dode *et al.* (2006) identified different point mutations in the prokineticin-2 gene (*PROK2*) gene and in its receptor, *PROKR2*, respectively [21]. The mutations in *PROK2* were detected in the heterozygous state, whereas *PROKR2* mutations were found in the heterozygous, homozygous, or compound heterozygous state. In addition, one of the patients heterozygous for a *PROKR2* mutation was also carrying a missense mutation in *KAL1*, thus indicating a possible digenic inheritance of the disease in this individual.
TAC3 and TACR3	IHH	Topaloglu *et al.* (2009) reported four human pedigrees with severe congenital gonadotropin deficiency and pubertal failure in which all

[OMIM#162330; 162332]		affected individuals were homozygous for loss-of-function mutations in *TAC3* (encoding Neurokinin B) or its receptor *TACR3* (encoding NK3R) [22].
WDR11 [OMIM#606417]	KS and IHH	Kim et al. (2010) screened 201 normosmic or hyposmic/anosmic HH patients for mutations in the *WDR11* gene and identified 5 different heterozygous missense mutations in 6 unrelated probands, including 5 normosmic patients and 1 anosmic patient [23].

Hypogonadotropic Hypogonadism Panel Sequencing Panel (19 genes sequencing)

Sample specifications: 3 to10 cc of blood in a purple top (EDTA) tube
Cost: $3500
CPT codes: 81407
Turn-around time: 8 weeks
Note: We cannot bill insurance for the above test.

Kallmann Syndrome Sequencing Panel (16 genes sequencing)

Sample specifications: 3 to10 cc of blood in a purple top (EDTA) tube
Cost: $3000
CPT codes: 81407
Turn-around time: 8 weeks
Note: We cannot bill insurance for the above test.

Test methods:

Comprehensive sequence coverage of the coding regions and splice junctions of all genes in this panel is performed. Targets of interest are enriched and prepared for sequencing using the Agilent SureSelect system. Sequencing is performed using Illumina technology and reads are aligned to the reference sequence. Variants are identified and evaluated using a custom collection of bioinformatic tools and comprehensively interpreted by our team of directors and genetic counselors. All pathogenic and likely pathogenic variants are confirmed by Sanger sequencing. The technical sensitivity of this test is estimated to be >99% for single nucleotide changes and insertions and deletions of less than 20 bp.

Results:

Results, along with an interpretive report, will be faxed to the referring physician. Additional reports will be provided as requested. All abnormal results will be reported by telephone.

For more information about our testing options, please visit our website at dnatesting.uchicago.edu or contact us at 773-834-0555.

References:

1. Seminara, S.B., F.J. Hayes, and W.F. Crowley, Jr., *Gonadotropin-releasing hormone deficiency in the human (idiopathic hypogonadotropic hypogonadism and Kallmann's syndrome): pathophysiological and genetic considerations.* Endocr Rev, 1998. **19**(5): p. 521-39.
2. Mitchell, A.L., et al., *Genetic basis and variable phenotypic expression of Kallmann syndrome: towards a unifying theory.* Trends Endocrinol Metab, 2011. **22**(7): p. 249-58.
3. Bhasin, S., et al., *Testosterone therapy in men with androgen deficiency syndromes: an Endocrine Society clinical practice guideline.* J Clin Endocrinol Metab, 2010. **95**(6): p. 2536-59.
4. Kim, H.G., et al., *Mutations in CHD7, encoding a chromatin-remodeling protein, cause idiopathic hypogonadotropic hypogonadism and Kallmann syndrome.* Am J Hum Genet, 2008. **83**(4): p. 511-9.
5. Kotan, L.D., et al., *Mutations in FEZF1 cause Kallmann syndrome.* Am J Hum Genet, 2014. **95**(3): p. 326-31.
6. Miraoui, H., et al., *Mutations in FGF17, IL17RD, DUSP6, SPRY4, and FLRT3 are identified in individuals with congenital hypogonadotropic hypogonadism.* Am J Hum Genet, 2013. **92**(5): p. 725-43.
7. Falardeau, J., et al., *Decreased FGF8 signaling causes deficiency of gonadotropin-releasing hormone in humans and mice.* J Clin Invest, 2008. **118**(8): p. 2822-31.
8. Dode, C., et al., *Loss-of-function mutations in FGFR1 cause autosomal dominant Kallmann syndrome.* Nat Genet, 2003. **33**(4): p. 463-5.
9. Sato, N., et al., *Clinical assessment and mutation analysis of Kallmann syndrome 1 (KAL1) and fibroblast growth factor receptor 1 (FGFR1, or KAL2) in five families and 18 sporadic patients.* J Clin Endocrinol Metab, 2004. **89**(3): p. 1079-88.
10. Matthews, C.H., et al., *Primary amenorrhoea and infertility due to a mutation in the beta-subunit of follicle-stimulating hormone.* Nat Genet, 1993. **5**(1): p. 83-6.
11. Phillip, M., et al., *Male hypogonadism due to a mutation in the gene for the beta-subunit of follicle-stimulating hormone.* N Engl J Med, 1998. **338**(24): p. 1729-32.
12. Lindstedt, G., et al., *Follitropin (FSH) deficiency in an infertile male due to FSHbeta gene mutation. A syndrome of normal puberty and virilization but underdeveloped testicles with azoospermia, low FSH but high lutropin and normal serum testosterone concentrations.* Clin Chem Lab Med, 1998. **36**(8): p. 663-5.
13. Bouligand, J., et al., *Isolated familial hypogonadotropic hypogonadism and a GNRH1 mutation.* N Engl J Med, 2009. **360**(26): p. 2742-8.

14. de Roux, N., et al., *A family with hypogonadotropic hypogonadism and mutations in the gonadotropin-releasing hormone receptor.* N Engl J Med, 1997. **337**(22): p. 1597-602.
15. Layman, L.C., et al., *Mutations in gonadotropin-releasing hormone receptor gene cause hypogonadotropic hypogonadism.* Nat Genet, 1998. **18**(1): p. 14-5.
16. Hardelin, J.P., et al., *Heterogeneity in the mutations responsible for X chromosome-linked Kallmann syndrome.* Hum Mol Genet, 1993. **2**(4): p. 373-7.
17. Silveira, L.G., et al., *Mutations of the KISS1 gene in disorders of puberty.* J Clin Endocrinol Metab, 2010. **95**(5): p. 2276-80.
18. Basciani, S., et al., *Hypogonadism in a patient with two novel mutations of the luteinizing hormone beta-subunit gene expressed in a compound heterozygous form.* J Clin Endocrinol Metab, 2012. **97**(9): p. 3031-8.
19. Golden, M.P., B.M. Lippe, and S.A. Kaplan, *Congenital adrenal hypoplasia and hypogonadotropic hypogonadism.* Am J Dis Child, 1977. **131**(10): p. 1117-8.
20. Xu, N., et al., *Nasal embryonic LHRH factor (NELF) mutations in patients with normosmic hypogonadotropic hypogonadism and Kallmann syndrome.* Fertil Steril, 2011. **95**(5): p. 1613-20 e1-7.
21. Dode, C., et al., *Kallmann syndrome: mutations in the genes encoding prokineticin-2 and prokineticin receptor-2.* PLoS Genet, 2006. **2**(10): p. e175.
22. Topaloglu, A.K., et al., *TAC3 and TACR3 mutations in familial hypogonadotropic hypogonadism reveal a key role for Neurokinin B in the central control of reproduction.* Nat Genet, 2009. **41**(3): p. 354-8.
23. Kim, H.G., et al., *WDR11, a WD protein that interacts with transcription factor EMX1, is mutated in idiopathic hypogonadotropic hypogonadism and Kallmann syndrome.* Am J Hum Genet, 2010. **87**(4): p. 465-79.