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The prevalence of digenic mutations in patients with normosmic hypogonadotropic hypogonadism and Kallmann syndrome

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Abstract

Objective—To determine the prevalence of digenic mutations in patients with idiopathic hypogonadotropic hypogonadism (IHH) and Kallmann syndrome (KS).

Design—Molecular analysis of DNA in IHH/KS patients.

Setting—Academic medical center.

Patient(s)—Twenty-four IHH/KS patients with a known mutation (group 1) and 24 IHH/KS patients with no known mutation (group 2).

Intervention(s)—DNA from IHH/KS patients was subjected to polymerase chain reaction–based DNA sequencing of the 13 most common genes (KAL1, GNRHR, FGFR1, KISS1R, TAC3, TACR3, FGF8, PROKR2, PROK2, CHD7, NELF, GNRH1, and *WDR11*).

Main Outcome Measure(s)—The identification of mutations absent in 188 ethnically matched controls. Both SIFT (sorting intolerant from tolerant) and conservation among orthologs provided supportive evidence for pathologic roles.

Result(s)—In group 1, 6 (25%) of 24 IHH/KS patients had a heterozygous mutation in a second gene, and in group 2, 13 (54.2%) of 24 had a mutation in at least one gene, but none had digenic mutations. In group 2, 7 (29.2%) of 24 had a mutation considered sufficient to cause the phenotype.

Conclusion(s)—When the 13 most common IHH/KS genes are studied, the overall prevalence of digenic gene mutations in IHH/KS was 12.5%. In addition, approximately 30% of patients without a known mutation had a mutation in a single gene. With the current state of knowledge, these findings suggest that most IHH/KS patients have a monogenic etiology.

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Keywords

Digenic mutations; idiopathic hypogonadotropic hypogonadism; Kallmann syndrome

The hypothalamic-pituitary-gonadal (HPG) axis plays a crucial role in the development and progression through puberty, and ultimately reproductive competence. This neuroendocrine axis is controlled by the decapeptide gonadotropin-releasing hormone (GnRH). Neurons of GnRH originate in the olfactory placode/vomerolateral organ region and migrate into the hypothalamus along olfactory neurons where they extend their processes to the median eminence (1, 2). The pulsatile secretion of GnRH into the hypophyseal-portal vessels controls the synthesis and release of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) in the anterior pituitary gland, which then stimulate the gonads to produce sex steroids and gametes. In the pubertal disorder idiopathic hypogonadotropic hypogonadism (IHH), GnRH secretion and/or action are impaired. Therefore, these patients have low sex steroids, low gonadotropins, and absent or disrupted puberty (3).

Idiopathic hypogonadotropic hypogonadism may be normosmic (nIHH) or it may be associated with anosmia, which is known as Kallmann syndrome (KS). Kallmann syndrome results when GnRH neuronal migration is halted within the meninges and GnRH neurons do not cross the cribriform plate; therefore, both GnRH and olfactory neurons do not reach the hypothalamus (4). In addition to reproductive dysfunction, IHH/KS patients may also manifest a variety of other nonreproductive disorders such as midline facial defects, dental agenesis, hearing loss, a variety of neurologic defects, and renal agenesis (3).

IHH/KS may be inherited as X-linked recessive, autosomal dominant, or autosomal recessive modes in addition to apparently sporadic forms. Mutations in at least 17 genes contribute to the molecular basis of IHH/KS, and they include *KAL1*, *NR0B1*, *GNRHR*, *FGFR1*, *KISS1R*, *TACR3*, *TAC3*, *FGF8*, *CHD7*, *PROKR2*, *PROK2*, *GNRH1*, *NELF*, *WDR11*, *PCSK1*, *LEP*, and *LEPR* (5). In addition, at least six genes are involved in combined pituitary hormone deficiency, which may also affect gonadotropes (5). However, these genes only account for approximately 30% of the etiologies of all IHH/KS patients.

Digenic mutations have been increasingly described in IHH/KS, although the prevalence is unknown. In 2006, Dode et al. (6) reported a patient who had mutations in two genes (*PROKR2* and *KAL1*). Since that time, a number of other investigators have described individual or several cases of digenic mutations in IHH/KS (7–12). In the largest study to date, eight genes (*FGFR1*, *KAL1*, *PROKR2*, *GNRHR*, *FGF8*, *KISS1R*, *NELF*, and *PROK2*) were analyzed in IHH/KS (13). The prevalence of digenic disease was about 11% in those IHH/KS patients who had a known mutation in one gene and 2.5% of all patients. However, *CHD7* and *WDR11*, which comprise 6% (14) and 3% (15), respectively, of IHH/KS mutations, were not included in this analysis. Therefore, the purpose of the present study was to determine the prevalence of digenic disease in IHH/KS patients by studying all of the most common genes.

MATERIALS AND METHODS

Patients

A total of 48 IHH/KS patients (31 males and 17 females) were studied for mutations in 13 IHH/KS genes. Twenty-four patients had one known mutation in an IHH/KS gene (group 1), and 24 IHH/KS patients had no known mutation (group 2). We defined IHH as either absent or incomplete pubertal development at age 17 in girls and 18 in boys, inappropriately low or normal levels of LH and FSH, and low sex steroids (hypoestrogenism in females and low

testosterone in males). Other causes were excluded as described previously (3). Complete IHH/KS was defined as the absence of puberty without thelarche (Tanner 1) in females and testes ≤ 3 mL in males. Incomplete IHH/KS was defined as partial breast development in females and testes ≤ 4 mL in males (3). Anosmia was defined using the University of Pennsylvania Smell Test, when available, or by history. White blood cells or lymphoblastoid cell lines were used as a source for DNA, RNA, and/or protein. All patients signed an informed consent approved by the Human Assurance Committee of the Georgia Health Sciences University.

The DNA was subjected to polymerase chain reaction (PCR) analysis and DNA sequencing for the protein coding exons and splice junctions of *KAL1*, *GNRHR*, *FGFR1*, *KISS1R*, *TAC3*, *TACR3*, *FGF8*, *PROKR2*, *PROK2*, *CHD7*, *NELF*, *GNRH1*, and *WDR11* genes. These genes were selected because they are the 13 most common of the known 17 IHH/KS genes. The primer sequences and PCR conditions have either been published previously (12, 14, 15) or may be provided upon request. Verification of preliminary sequence data was performed by repeat PCR, DNA sequencing, and the use of BLAST (Basic Local Alignment Search Tool) and the single-nucleotide polymorphism (SNP) database. Family members were studied for segregation of mutations when available. Each mutation was tested in 188 ethnically matched controls.

Missense mutations were analyzed in silico with SIFT (sorting intolerant from tolerant) (16, 17) using all orthologs with at least 80% homology to the human protein. Causative mutations absent in 188 controls were defined as frameshift, splicing, or missense if confirmed by at least one in vitro method. Probable mutations included missense mutations that were absent in 188 controls, had high conservation among other orthologs, and/or were intolerant by SIFT. A mutation was considered possible if it was not found in 188 controls but was considered tolerant by SIFT or was not predicted to affect splicing. If the nucleotide sequence was present in both patients and controls, it was considered a polymorphism.

To predict potential effects upon on 5' and 3' splicing consensus sequences, the sequence was analyzed at the Berkley Drosophila Genome Project site (http://www.fruitfly.org/seq_tools/splice). Putative mutations located in introns near splice sites were analyzed using ESE Finder Version 3 (<http://rulai.cshl.edu/cgi-bin/tools/ESE3/esefinder.cgi>) to determine any effect upon predicted SR protein-binding sites involved in splicing (18).

RESULTS

Both groups of patients were sequenced for mutations in 13 genes: *KAL1*, *GNRHR*, *FGFR1*, *KISS1R*, *TAC3*, *TACR3*, *FGF8*, *PROKR2*, *PROK2*, *CHD7*, *NELF*, *GNRH1*, and *WDR11* genes. The mutations in the 24 patients with one known mutation included hemizygous mutations in *KAL1* (n = 4); heterozygous mutations in *WDR11* (n = 7), *CHD7* (n = 6), *NELF* (n = 1), and *FGFR1* (n = 2); or biallelic mutations in *GNRHR* (n = 3) or *NELF* (n = 1). One of the *WDR11* heterozygous mutations was considered a rare sequence variant (RSV). Electropherograms of the characterized mutations are shown in Supplemental Figure 1 (available online). Of the 24 patients with a known mutation (group 1), 7 (29.2%) had a mutation or probable mutation in a second gene. The seven mutations included heterozygous *TACR3* (n = 2), hemizygous *KAL1* (n = 3), and homozygous *GNRHR* (n = 1) (Table 1A). One patient with a *KAL1* mutation had heterozygous mutations in two genes—*NELF* and *PROK2*. Six of the seven patients who had mutations in two or more genes were male and included five KS patients and two nIHH patients (Supplemental Table 1, available online).

None of the mutations in these genes were present in the SNP database or observed in 188 to 192 controls. Three of the seven mutations found in group 1 have been previously reported in IHH/KS patients: *NELF* p.A253T (12), *TACR3* p.W275X (12), and *KAL1* p.V587L (13), and four had not. The *TACR3* nonsense mutation (p.W275X) was found in two different patients in group 1. It is likely that six of the seven nucleotide changes are mutations, but the *KAL1* S511Y is predicted to be tolerated by SIFT and is therefore likely a polymorphism. Therefore, 6 (25%) of 24 patients with a previous mutation had what were considered true or probable second mutations (Fig. 1).

Of the 24 individuals without a known mutation, 13 (54.1%) had a nucleotide change in one gene (Table 1B) that was not seen in the SNP database or controls. No patient from group 2 had mutations in two genes, although patient 12 in Table 1B had a *KAL1* intronic RSV that is not predicted to affect splicing. Mutations occurred in *KAL1*, *PROKR2*, *GNRHR*, *TACR3*, *GNRHI*, and *FGFR1* in patients from this group. All of these nucleotide changes were heterozygous or hemizygous except the compound heterozygous *PROKR2* (p.V551/c.57delC) mutations in patient 4, compound heterozygous *TACR3* (p.W275X/A91E) mutations in patient 5, and compound heterozygous *GNRHR* (R262Q/L266R) in patient 12 (see Table 1B). All 13 patients with nucleotide changes in a second gene had true or probable mutations (see Table 1B).

Of the 13 heterozygous mutations from group 2, 6 of 13 only occurred in one allele in known autosomal recessively inherited forms (*PROKR2*, *TACR3*, and *GNRHI*), indicating that these patients demonstrated only carrier status for these particular genes rather than causation. Patient 12 had compound heterozygous *GNRHR* mutations and a hemizygous *KAL1* intron mutation, which is present in both parents, indicating that it is a polymorphism. Therefore, considering both groups 1 (6 of 24) and 2 (0 of 24), a total of 6 (12.5%) of 48 had true or probable mutations in two or more genes (see Fig. 1). Of the 10 new missense mutations identified, nine were predicted deleterious by SIFT (Supplemental Table 2, available online), and all were highly conserved among species (Supplemental Fig. 2, available online). Of note, 6 (12.5%) of 48 IHH/KS patients had heterozygous (n = 5) or compound heterozygous (n = 1) *TACR3* mutations. Three of these have not been reported: p.A91E, p.R364Q, and p.R441C (see Table 1B). One patient had a heterozygous *GNRHI* mutation (p.R31C) that disrupted the eighth amino acid of the decapeptide and had been reported previously in the heterozygous state (10).

Of all identified mutations (the second gene mutation in group 1 and all of the mutations found in group 2), 11 had available family members who were studied further (Fig. 2). Familial segregation occurred as expected for the *KAL1* intron mutation (patient 11 in group 2; see Fig. 2A), being present in the carrier mother and affected son, as well as for the *GNRHR* mutations (patient 12 in group 2) in which each parent was heterozygous for a different allele while the proband demonstrated compound heterozygosity (see Fig. 2B). For patient 21 in group 1 who had a heterozygous *FGFR1* D768H mutation, both his unaffected father and unaffected brother had the same mutation (this patient had no mutation in a second gene). For the remainder of the families, segregation occurred as expected, but having only one allele of an autosomal recessive disease does not explain the molecular basis of the phenotype (see Fig. 2D–F, H, and J). In two cases of *KAL1* mutations, the mother's sample was not available to determine carrier status (see Fig. 2G and I); in one case of *KAL1*, both parents carried the same nucleotide change (see Fig. 2K), indicating a polymorphism.

DISCUSSION

Mutations in at least 17 genes have been shown to be involved in the pathophysiology of IHH/KS, but the mode of inheritance is not completely known for all genes because of the paucity of described mutations. However, current evidence suggests X-linked recessive (*KAL1*, *NR0B1*), autosomal dominant (*FGFR1*, *FGF8*, *CHD7*, *WDR11*), and autosomal recessive (*GNRHR*, *KISS1R*, *GNRH1*, *TACR3*, *TAC3*, *PROKR2*, *PROK2*, *LEP*, *LEPR*, and *PCSK1*) inheritance patterns. *NELF* is likely to be autosomal recessive because biallelic mutations, reducing protein expression in vitro, have only been described in one KS patient without a mutation in another gene (12), whereas heterozygous *NELF* mutations have only been found in affected IHH/KS patients with heterozygous mutations in another gene (9, 12). Mutations in *LEP*, *LEPR*, and *PCSK1* were not tested in the current study because of their rarity (19); and *NR0B1* was not tested because mutations are extremely rare unless there is coexistent adrenal failure (20).

Mutations in more than one gene have been reported for a number of genes in IHH/KS. Dode et al. (6) first described heterozygous *PROKR2* and hemizygous *KAL1* mutations in a KS patient; and Pitteloud et al. (9) described heterozygous *FGFR1/NELF* as well as *FGFR1/GNRHR* mutations. Although either the heterozygous *FGFR1* or biallelic *GNRHR* mutations are capable of causing disease without the involvement of another gene, these investigators showed that KS did not result unless the patient had both genes involved (9). Since that time, other digenic patterns have been reported—*FGFR1/FGF8*, *PROK2/PROKR2*, *FGFR1/PROKR2*, *NELF/KAL1*, and *NELF/TACR3* (Table 2). In the present study involving screening the 13 most common genes involved in IHH/KS, we have identified three new digenic combinations of *WDR11/KAL1*, *WDR11/GNRHR*, and *KAL1/TACR3* as well as the novel trigenic pattern of *KAL1/NELF/PROKR2* (see Table 2).

It is interesting that 12.5% of our 48 IHH/KS patients had at least one mutant *TACR3* allele, five with heterozygous (10.4%) and one with compound heterozygous mutations (2.1%). In a previous report of 345 patients analyzed for *TACR3*, 6 (1.7%) of 345 and 5 (1.4%) of 345 demonstrated biallelic (homozygous or compound heterozygous) and heterozygous mutations, respectively (21). Our approximately 2% rate of biallelic mutations is comparable with that reported previously elsewhere (21); because *TACR3* mutations are inherited in an autosomal recessive fashion, two mutant alleles are sufficient to cause disease. Three of our patients had heterozygous *TACR3* mutations without mutations in another gene, whereas two had heterozygous *TACR3* mutations with either a hemizygous *KAL1* or *NELF* mutation. Four of our six patients with *TACR3* mutations had a heterozygous p.W275X allele, which appears to be the most commonly reported *TACR3* mutation (21) and suggests that it represents a potential hotspot for mutations.

As shown in Table 2, we now add four new types of digenic/trigenic disease in five patients to the previously reported 13 cases in the literature, bringing the total to 18. There are several interesting observations concerning the reported IHH/KS patients with digenic mutations. First, for 17 of the 18 patients, a mutation in one of the genes is sufficient to cause IHH/KS based upon the presence of hemizygous *KAL1* (X-linked recessive), heterozygous *FGFR1*, *FGF8*, and *WDR11* (autosomal dominant), or biallelic *GNRHR* (autosomal recessive) mutations. It is interesting that patient 6 in Table 2 had compound heterozygous *FGFR1* mutations and homozygous *FGF8* mutations. The only patient with digenic mutations in which heterozygous mutations of one gene was not sufficient to explain the phenotype in patient 5 in Table 2 who was heterozygous for *PROK2/PROKR2* mutations, both of which are normally autosomal recessive (22).

The prevalence of digenic mutations in IHH/KS has not been extensively reported, being mostly presented as single cases. However, Sykiotis et al (13) published the largest series in which they studied eight genes (*FGFR1*, *KAL1*, *PROKR2*, *GNRHR*, *FGF8*, *KISS1R*, *NELF*, and *PROK2*) in 397 IHH/KS patients and found digenic disease in 10 (11%) of 88 patients who had a known mutation in one gene and 10 (2.5%) of 397 among all patients. We have extended the analysis to the 13 most common IHH/KS genes (*KAL1*, *GNRHR*, *FGFR1*, *KISS1R*, *TAC3*, *TACR3*, *FGF8*, *PROKR2*, *PROK2*, *CHD7*, *NELF*, *GNRH1*, and *WDR11*), including the common *CHD7* (14) and *WDR11* (15) genes. Our findings indicate that 6 (25%) of 24 patients with one known mutation (group 1) had a mutation in a second gene, which is double that of Sykiotis et al. (13), most likely because of the addition of five more genes being studied. However, 0 of 24 patients with no known mutation had digenic mutations, which is in the same range as the 2.5% reported by Sykiotis et al. (13). In total, this constitutes 6 (12.5%) of 48 of all patients in our study who had digenic disease. When just the patients without an existing mutation were considered, DNA sequencing of these 13 most common IHH/KS genes resulted in a likely etiology in approximately 30%. Therefore, most patients appear to have monogenic IHH/KS, given the current number of genes being studied.

Just because mutations in more than one gene have been identified in some IHH/KS patients, this does not prove that they have a causative role in the phenotype. However, it is very possible that less severe mutations in two or more genes (for example, heterozygous mutations in genes that normally cause autosomal recessive disease) could function within the same pathway and result in disease by synergistic heterozygosity (12, 23–25). There is precedent for synergistic heterozygosity in human diseases of primary pulmonary hypertension and metabolic disorders (23–25). It is quite conceivable and likely that many of the known IHH/KS genes function in this manner, as evidenced by the interaction of *FGF8*, *FGFR1*, and *KAL1*. *FGF8* is a ligand for *FGFR1* (8); and it is known that both *FGFR1* and *KAL1*-encoded anosmin-1 proteins use heparin sulfate in their action (26). Digenic disease has been reported in other genetic diseases as well, including Bardet-Biedl syndrome (27), epidermolysis bullosa (28), and retinitis pigmentosa (29).

Of 48 IHH/KS patients screened for mutations in 13 of the most commonly involved genes, 6 (12.5%) of 48 had mutations in two or more genes. We realize that this sample size is not large and that future studies are needed to clarify the role of digenic mutations and the pathways involved, but our findings and those of others serve to suggest which genes could interact with each other. For example, if a patient has digenic mutations that result in the IHH/KS phenotype, it is tempting to speculate that those particular genes (such as *KAL1/TACR3* or *KAL1/NELF/PROK2*) could be intimately involved in the same developmental pathway by synergistic heterozygosity. Nevertheless, our findings from the present study indicate that just under 90% of all IHH/KS patients possess mutations in a single gene, indicating that monogenic mutations account for most cases of IHH/KS.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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FIGURE 1.

An overview of the study protocol and findings showing patients with idiopathic hypogonadotropic hypogonadism and Kallmann syndrome (IHH/KS) with a known mutation (group 1) and those without a mutation (group 2).

FIGURE 2.

Pedigrees for 11 patients with idiopathic hypogonadotropic hypogonadism and Kallmann syndrome (IHH/KS) with mutations who have available family members for segregation analysis. *Squares* indicate males, *circles* indicate females, *completely shaded circles* indicate affected individuals, *half-shaded circles* or *squares* indicate carriers of recessive diseases, and *circles with a dot* represent carriers of X-linked recessive disease. *Arrows* point to the proband. Known genotypes are indicated below the individual (if an individual has no genotype listed, then DNA was not available).

TABLE 1A

Patients with idiopathic hypogonadotropic hypogonadism (IHH) and Kallmann syndrome (KS) with one mutation (group 1).

Patient	Phenotype	Known mutation	Functional effect	2nd mutation	Functional effect	Mutation
1	KS/M	<i>KAL1</i> [c.491-493delGTT; p.C164del] (30)	Predicted protein misfolding	<i>NELF</i> [c.757G>A; p.A253T] (12) <i>PROK2</i> [c.122 G>T; p.G41D]	<i>NELF</i> : Decreased protein expression <i>PROK2</i> : Conserved; SIFT intolerant	TM PM
2	KS/M	<i>KAL1</i> [c.769C>T; p.R257X] (30, 31)	NMD or protein truncation	<i>TACR3</i> [c.824G>A; p.W275X] (12, 21)	PTC	TM
3	KS/M	<i>NELF</i> [c.1160-13C>T] (12)	Causes exon skipping	<i>TACR3</i> [c.824G>A; p.W275X] (12, 21)	PTC	TM
4	IHH/F	<i>GNRHR</i> [c.785G>A; p.R262Q] (32, 33) <i>GNRHR</i> [c.851 A>G; p.Y284C] (33)	Both decrease receptor expression and signaling	<i>KAL1</i> [c.1532 C>A; p.S511Y]	SIFT: predicted tolerant	SNP
5	KS/M	<i>WDR11</i> [c.2070T>A; p.H690Q] (15)	Abolish EMX1 binding; Conserved; SIFT intolerant	<i>KAL1</i> [c.490T>C; p.C164R]	Conserved; SIFT intolerant	PM
6	KS/M	<i>WDR11</i> [c.2932A>C; p.K978Q] (15)	Conserved; SIFT intolerant RSV since seen in 1/587 controls	<i>KAL1</i> [c.1759 G>T; p.V587L] (13)	Conserved; SIFT intolerant	PM
7	IHH/M	<i>WDR11</i> [c.1303G>A; p.A435T] (15)	Abolished EMX1 binding Conserved; SIFT intolerant	<i>GNRHR</i> [c.275T>C; p.L92P]	Conserved; SIFT intolerant	PM
8	IHH/M	<i>CHD7</i> [c.8842A>G; p.K2948E] (14)	Conserved; SIFT intolerant	-	-	-
9	IHH/M	<i>WDR11</i> [c.3450T>G; p.F1150L] (15)	Conserved	-	-	-
10	KS/M	<i>KAL1</i> [c.490T>C; p.C164R]	Conserved; SIFT intolerant	-	-	-
11	IHH/M	<i>NELF</i> [c.629-21G>C; c.629-23C>G] (12)	Decreased protein expression	-	-	-
12	IHH/M	<i>CHD7</i> [c.2501C>T; p.S834F] (14)	Conserved; SIFT intolerant	-	-	-
13	IHH/M	<i>WDR11</i> [c.1183C>T; p.R395W] (15)	Conserved; SIFT intolerant	-	-	-
14	IHH/F	<i>WDR11</i> [c.1343G>A; p.R448Q] (15)	Destabilizes WDR11 dimer and impairs binding	-	-	-
15	IHH/F	<i>WDR11</i> [c.3450T>G; p.F1150L] (15)	Conserved; SIFT intolerant	-	-	-
16	IHH/F	<i>GNRHR</i> [c.317 A>G; p.Q106R] (32, 34) <i>GNRHR</i> [c.797T>G; p.L266R] (35)	Decreased binding and activation of intracellular signaling ^{a,b}	-	-	-
17	IHH/M	<i>GNRHR</i> ^a [c.386C>A; p.A129D] (32, 36) <i>GNRHR</i> ^b [c.785G>A; p.R262Q] (33)	Decreased binding and IP3 signaling Decreased receptor expression and signaling ^b	-	-	-
18	KS/M	<i>CHD7</i> [c.8639 C>T; p.P2880L] (14)	Conserved; SIFT intolerant	-	-	-
19	KS/M	<i>CHD7</i> [c.164A>G; p.H55R] (14)	Conserved; SIFT intolerant	-	-	-
20	KS/F	<i>CHD7</i> [IVS6+5G>C] (14)	Exon skipping	-	-	-
21	IHH/F	<i>FGFR1</i> [c.2302 G>C; p.D768H] (13)	SIFT intolerant	-	-	-

Patient	Phenotype	Known mutation	Functional effect	2nd mutation	Functional effect	Mutation
22	KS/F	<i>FGFR1</i> [c.301T>G; p.C101G]	Conserved; SIFT intolerant	–	–	–
23	KS/M	<i>KAL1</i> [c.769C>T; p.R257SX] (30, 31)	NMD or protein truncation	–	–	–
24	IHH/M	<i>CHD7</i> [c.8365G>A; p.A2789T] (14)	SIFT intolerant	–	–	–

Note: Both the cDNA sequence (indicated by c.) and protein sequence (indicated by p.) affected by the mutation are shown. Previously reported mutations are referenced. Patients 1 to 7 had heterozygous mutations in a second gene. The first seven patients (except no. 4) had mutations either predicted to be deleterious or previously reported. M = male; F = female; HH = hypogonadotropic hypogonadism; NMD = nonsense mediated decay; PTC = premature termination codon; PM = probable mutation; SIFT = sorting intolerant from tolerant; SNP = single-nucleotide polymorphism; TM = true mutation; RSV = rare sequence variant; HH = hypogonadotropic hypogonadism with unknown sense of smell status.

TABLE 1B

Patients with idiopathic hypogonadotropic hypogonadism (IHH) and Kallmann syndrome (KS) of the 24 total who did not have a mutation before screening (group 2).

Patient	Disease/sex	Putative mutation	Function	Mutation	Likely sufficient to cause disease
1	KS/M	<i>FGFR1</i> [c.2059 G>A; p.G687R] (37)	Located in tyrosine kinase domain 2 (TK2); Conserved; SIFT intolerant	PM	Yes
2	KS/M	<i>KAL1</i> [IVS7+5G>A]	Predicted to affect splicing	PM	Yes
3	IHH/M	<i>TACR3</i> [c.824G>A; p.W275X] (12, 21)	Intracellular/cytoplasmic loop, PTC	TM	No (heterozygous)
4 ^a	KS/M	<i>PROKR2</i> [c.163 G>A; p.V55I] <i>PROKR2</i> [c.57delC]	Conserved; SIFT intolerant	PM TM	Yes
5 ^a	IHH/M	<i>TACR3</i> [c.272 C>A; p.A91E] <i>TACR3</i> [c.824G>A; p.W275X] (12, 21)	Conserved; SIFT intolerant PTC	PM TM	Yes
6	IHH/F	<i>PROKR2</i> [c.518 T>G; p.L173R] (6, 7, 22, 38)	Impaired calcium signaling activity, cell surface targeting defect; impaired stability and correct folding of the receptor	TM	No (heterozygous)
7	IHH/F	<i>PROKR2</i> [c.491G>A; p.R164Q] (6, 7, 38)	Conserved; SIFT intolerant	PM	No (heterozygous)
8	IHH/F	<i>TACR3</i> [c.1091 G>A; p.R364Q]	Conserved; SIFT intolerant	PM	No (heterozygous)
9	KS/M	<i>KAL1</i> [c.1870_1871insG]	Frameshift	TM	Yes
10	KS/F	<i>TACR3</i> [c.1321C>T; p.R441C]	Conserved; SIFT intolerant	PM	No (heterozygous)
11	KS/M	<i>KAL1</i> [IVS7+5G>C]	Predicted to affect splicing	PM	Yes
12 ^a	IHH/F	<i>GNRHR</i> [c.785G>A; p.R262Q] (32, 33)	Decreased receptor expression and decreased binding and intracellular signaling	TM	Yes
		<i>GNRHR</i> * [c.797T>G; p.L266R]	Conserved; SIFT intolerant	PM	
13	HH/F	<i>KAL1</i> [IVS14+26G>T]	Present in both parents	SNP	Polymorphism
		<i>GNRH1</i> [c.93 c>T; p.R31C] (10)	Conserved; SIFT intolerant	PM	No (heterozygous)

Note: Only those patients with mutations are shown. (Patients 4, 5, and 12 had biallelic mutations.) F = female; M = male; PTC = premature termination codon; PM = probable mutation; SIFT = sorting intolerant from tolerant; SNP = single-nucleotide polymorphism; TM = true mutation.

^aNonheterozygous. All others are heterozygous.

TABLE 2

Reported digenic cases and the new cases identified from the present study.

Patient no.	Sex and phenotype	Gene 1	No. of alleles	Gene 2	No. of Alleles	Study
1	Male, KS	<i>KAL1</i>	1	<i>PROKR2</i>	1	Dode et al. (6)
2	Male, KS	<i>KAL1</i>	1	<i>PROKR2</i>	1	Canto et al. (39)
3	Male, KS	<i>FGFR1</i>	1	<i>NELF</i>	1	Pitteloud et al. (9)
4	Female, nIHH	<i>FGFR1</i>	1	<i>GNRHR</i>	2	Pitteloud et al. (9)
5	Female, KS	<i>PROK2</i>	1	<i>PROKR2</i>	1	Cole et al. (7)
6	Male, nIHH	<i>FGFR1</i>	2 (CPD HET)	<i>FGF8</i>	2 (HMZ)	Falardeau et al. (8)
7	Male, nIHH	<i>FGFR1</i>	1	<i>FGF8</i>	1	Falardeau et al. (8)
8	Female, nIHH	<i>FGFR1</i>	1	<i>GNRHR</i>	2	Raivio et al. (11)
9	Female, nIHH	<i>FGFR1</i>	1	<i>PROKR2</i>	1	Raivio et al. (11)
10	Male, KS	<i>NELF</i>	1	<i>TACR3</i>	1	Xu et al. (12)
11	Female, nIHH	<i>FGFR1</i>	1	<i>KAL1</i>	1	Syktotis et al. (13)
12	Male, KS	<i>FGFR1</i>	1	<i>FGF8</i>	1	Syktotis et al. (13)
13	Male, KS	<i>FGFR1</i>	1	<i>NELF</i>	1	Syktotis et al. (13)
14	Male, KS	<i>KAL1</i>	1	<i>NELF/PROK2</i>	1/1	Case 1 (present study)
15	Male, KS	<i>KAL1</i>	1	<i>TACR3</i>	1	Case 2 (present study)
16	Male, KS	<i>WDR11</i>	1	<i>KAL1</i>	1	Case 5 (present study)
17	Male, nIHH	<i>WDR11</i>	1	<i>KAL1</i>	1	Case 6 (present study)
18	Male, nIHH	<i>WDR11</i>	1	<i>GNRHR</i>	1	Case 7 (present study)

Note: CPD HET = compound heterozygous; HMZ = homozygous; KS = Kallmann syndrome; nIHH = normosmic idiopathic hypogonadotropic hypogonadism.