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

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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 $\geq$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. (Fertil Steril® 2011;96:1424–30. ©2011 by American Society for Reproductive Medicine.)

Key Words: 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/vomeronasal 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

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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	*KAL1* [c.491–493delGTT; p.C164del] (30)	Predicted protein misfolding	*NELF* [c.757G>A; p.A253T] (12) *PROK2* [c.122 G>T; p.G41D]	*NELF*: Decreased protein expression *PROK2*: Conserved; SIFT intolerant	TM PM
2	KS/M	*KAL1* [c.769C>T; p.R257X] (30, 31)	NMD or protein truncation	*TACR3* [c.824G>A; p.W275X] (12, 21)	PTC	TM
3	KS/M	*NELF* [c.1160-13C>T] (12)	Causes exon skipping	*TACR3* [c.824G>A; p.W275X] (12, 21)	PTC	TM
4	IHH/F	*GNRHR* [c.785G>A; p.R262Q] (32, 33) *GNRHR* [c.851 A>G; p.Y284C] (33)	Both decrease receptor expression and signaling	*KAL1* [c.1532 C>A; p.S511Y]	SIFT: predicted tolerant	SNP
5	KS/M	*WDR11* [c.2070T>A; p.H690Q] (15)	Abolish EMX1 binding; Conserved; SIFT intolerant	*KAL1* [c. 490T>C; p.C164R]	Conserved; SIFT intolerant	PM
6	KS/M	*WDR11* [c.2932A>C; p.K978Q] (15)	Conserved; SIFT intolerant RSV since seen in 1/587 controls	*KAL1* [c.1759 G>T; p.V587L](13)	Conserved; SIFT intolerant	PM
7	IHH/M	*WDR11* [c.1303G>A; p.A435T] (15)	Abolished EMX1 binding Conserved; SIFT intolerant	*GNRHR* [c.275T>C; p.L92P]	Conserved; SIFT intolerant	PM
8	IHH/M	*CHD7* [c.8842A>G; p.K2948E] (14)	Conserved; SIFT intolerant	–	–	
9	IHH/M	*WDR11* [c.3450T>G; p.F1150L] (15)	Conserved	–	–	
10	KS/M	*KAL1* [c.490T>C; p.C164R]	Conserved; SIFT intolerant	–	–	
11	IHH/M	*NELF* [c.629–21G>C; c.629-23C>G] (12)	Decreased protein expression	–	–	
12	IHH/M	*CHD7* [c.2501C>T; p.S834F] (14)	Conserved; SIFT intolerant	–	–	
13	IHH/M	*WDR11* [c.1183C>T; p.R395W] (15)	Conserved; SIFT intolerant	–	–	
14	HH/F	*WDR11* [c.1343G>A; p.R448Q] (15)	Destabilizes WDR11 dimer and impairs binding	–	–	
15	IHH/F	*WDR11* [c.3450T>G; p.F1150L] (15)	Conserved; SIFT intolerant	–	–	
16	IHH/F	*GNRHR* [c.317 A>G; p.Q106R] (32, 34) *GNRHR* [c.797T>G; p.L266R] (35)	Decreased binding and activation of intracellular signaling[a,b]	–	–	
17	IHH/M	*GNRHR*[a] [c.386C>A; p.A129D] (32, 36) *GNRHR*[b] [c.785G>A; p.R262Q] (33)	Decreased binding and IP_3 signaling[a] Decreased receptor expression and signaling[b]	–	–	
18	KS/M	*CHD7* [c.8639 C>T; p.P2880L] (14)	Conserved; SIFT intolerant	–	–	
19	KS/M	*CHD7* [c.164A>G; p.H55R] (14)	Conserved; SIFT intolerant	–	–	
20	KS/F	*CHD7* [IVS6+5G>C] (14)	Exon skipping	–	–	
21	IHH/F	*FGFR1* [c.2302 G>C; p.D768H] (13)	SIFT intolerant	–	–	
22	KS/F	*FGFR1* [c.301T>G; p.C101G]	Conserved; SIFT intolerant	–	–	
23	KS/M	*KAL1* [c.769C>T; p.R257SX] (30, 31)	NMD or protein truncation	–	–	
24	IHH/M	*CHD7* [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.

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