The Importance of Autosomal Genes in Kallmann Syndrome: Genotype-Phenotype Correlations and Neuroendocrine Characteristics*

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ABSTRACT

Kallmann syndrome (KS) consists of congenital, isolated, idiopathic hypogonadotropic hypogonadism (IHH) and anosmia. The gene responsible for the X-linked form of KS, KAL, encodes a protein, anosmin, that plays a key role in the migration of GnRH neurons and olfactory nerves to the hypothalamus. In addition to X-linked pedigrees, autosomal dominant and recessive kindreds with KS have been reported. The relative importance of these autosomal vs. X-linked genes in producing KS, and the frequency of KAL mutations, are currently unknown because these are rare disorders and large series are unusual.

We examined 101 individuals with IHH (± anosmia) and their families to determine their modes of inheritance, incidence of mutations in the coding sequence of KAL, genotype-phenotype correlations, and [in a subset (n = 38)] their neuroendocrine phenotype.

Of the 101 patients, 59 had true KS (IHH + anosmia/hyposmia); whereas, in the remaining 42, no anosmia was evident in the patients or their families. Of the 59 KS patients, 21 were familial, whereas 38 were sporadic cases. Mutations in the coding sequence of KAL were identified in only 3 of 21 familial cases (14%) and 4 of 38 (11%) of the sporadic cases. Of the X-linked cases confirmed by mutational analysis, only 1 of 3 pedigrees appeared X-linked by inspection whereas the other 2 contained only affected brothers. Female members of families to determine their modes of inheritance, incidence of mutations in the coding sequence of KAL, genotype-phenotype correlations, and [in a subset (n = 38)] their neuroendocrine phenotype.

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In 1944, KALLMANN first described the clinical association of hypogonadotropic hypogonadism and anosmia (1) that has been classified as Kallmann syndrome (KS) ever since. In 1992, the first genetic defect in the KAL gene in Xp 22.3 was described (2); and the physiology and developmental biology of the protein it encodes, anosmin, is undergoing characterization (3). In the X-linked form, KS is characterized by a failure of hypothalamic GnRH secretion caused by a migratory defect of GnRH neurons from the olfactory placode to the hypothalamus (4). In addition, the associated anomalies of unilateral renal agenesis and synkinesia have been suggested to be specific to the X-linked form (5–7). These phenotypic abnormalities seem to have correlates in the developmental pattern of KAL gene transcripts in the chick embryo (8).

However, in the human, KS is characterized by considerable clinical and genetic heterogeneity. Families have been documented with individuals having full KS, normosmic idiopathic hypogonadotropic hypogonadism (IHH), and isolated anosmia within a single sibship (9). To shed further light on this heterogeneity, we examined a broad spectrum of patients with IHH, with and without anosmia, to deter-
mine their clinical spectrum, modes of inheritance, incidence of mutations in the coding sequence of KAL, and neuroendocrine phenotypes. Our results demonstrate that autosomal genes are clearly responsible for the majority of both familial and sporadic KS and that these cases have clinical and neuroendocrine phenotypes that distinguish them from the X-linked form.

**Subjects and Methods**

**Experimental subjects**

One hundred and one probands with IHH were evaluated. In all patients, complete IHH was documented based on the following previously published criteria: age more than 18 yr; clinical signs and symptoms of hypogonadism; prepubertal testosterone (<100 ng/dL) and estradiol levels (<20 pg/mL); low or inappropriately normal gonadotropin levels; normal baseline and reserve testing of other anterior pituitary hormones; and normal radiological imaging of the hypothalamic-pituitary region (10). Patients were questioned as to their ability to smell, because the subjective reporting of anosmia has proven a reliable marker of this symptom (9).

**Modes of inheritance.** Genetic criteria were used to establish the likely mode of disease transmission as outlined in previous analyses (9). A family was classified as X-linked if only males were affected and unaffected females could be considered carriers. There could be no male-to-male transmission. A family was considered autosomal dominant if direct transmission of the phenotype was demonstrable across generations. Male-to-male transmission was considered definitive evidence for dominant inheritance. A family was classified as autosomal recessive if all affected individuals were members of the same generation and included at least one female. Consanguinity provided additional support for this designation. Delayed puberty and isolated anosmia were used as surrogate markers of the phenotype (9).

Despite these basic genetic criteria, it is not always possible to correctly distinguish between different modes of genetic transmission. For example, a nonpenetrant, dominant genetic trait may be expressed in a male grandparent and a male child but not in a female parent, thereby appearing X-linked. In addition, heterozygous female carriers with partial expression have been documented in numerous X-linked disorders. Finally, kindreds with only affected brothers within a single sibship may represent any of the major modes of genetic inheritance, including dominant, recessive, and X-linked forms (for the purposes of this study, these latter kindreds were labeled as indeterminate). Therefore, recognizing these ambiguities, the best effort was made to classify families appropriately, with the knowledge that the KAL mutation analysis performed in this study would provide additional clarification.

**Methods and materials**

**Mutation detection.** Mutation detection was carried out by multiple methods of analysis. In the first method, a sequence polymorphism, located 8.7 kb downstream from the 3’ end of the KAL gene, was used (11) to examine the possibility of association of this locus with IHH in affected individuals of four families. Other DNA samples were analyzed using temperature gradient gel electrophoresis (TGGE) or single-stranded conformational polymorphism (SSCP) on PCR products generated with exon-specific primers based on adjacent intronic sequences. All abnormalities discovered by TGGE and SSCP screening were confirmed by direct sequencing.

Genomic DNA was extracted from peripheral blood leukocytes using standard procedures (12). PCR oligonucleotide primers were based on those published by Hardelin et al. (5) or designed using Lasergene software (DNASTAR Inc., Madison, WI). The latter primers are: exon 1: 5’CGCGGCGGCAGCAGCCC-AGAAAAAGAC3’ (forward) and 1: 5’TCTTCCCTTATGCAGCCTGTTACTG3’ (reverse); exon 2: 5’CTTCCCTGATGCTTCTGATCTA3’ (forward) and 2: 5’CTTCCCTGATGCTTCTGATCTA3’ (reverse); exon 3: 5’ACCTTGAGTTGCGATACTG3’ (reverse) and 3: 5’AGGAAGGGTAAATGAGAAAAG3’ (forward); exon 4: 5’AGGAAGGGTAAATGAGAAAAG3’ (forward) and 4: 5’TCTTCCCTGATGCTTCTGATCTA3’ (reverse); exon 5: 5’TCTTCCCTGATGCTTCTGATCTA3’ (reverse) and 5’TCTTCCCTGATGCTTCTGATCTA3’ (forward). A 40-base GC-clamp was incorporated on the 5’-end of either the forward or reverse primer to increase the sensitivity of the TGGE technique (13, 14).

Before TGGE, the theoretical melting temperature of the predicted PCR product corresponding to each pair of primers was determined using WinMelt software (Bio-Rad Laboratories, Inc., Hercules, CA). These predictions allowed the optimization of primer design for each particular exon. Exons 7, 9, and 11 were each divided into two overlapping segments (i.e., exons 7.1 and 7.2) that were independently amplified in different reactions because computer analyses indicated that these full-length exons would not yield the uniform melting curves required for TGGE.

PCR reactions were performed in a vol of 100 μL containing 50 pmol of each PCR primer, 200 μmol of each deoxynucleotide triphosphate, 2.5U Taq polymerase, 10 mmol/L Tris-HCl (pH 8.3), 1.5 mmol/L MgCl2, 50 mmol/L KCl, 0.01% gelatin, and 200 ng genomic DNA. Amplification of exon 1 was carried out in the presence of 8% dimethylsulfoxide. PCR was performed in a Gene Amp 9600 system (Perkin-Elmer Corp., Foster City, CA) using 35 cycles. DNA was denatured at 95 C for 5 min in the first cycle and for 30 sec in all subsequent cycles. Annealing was performed at 53–69.7 C for 30 sec, depending on the exon. Elongation was performed at 72 C for 30 sec, with a final elongation of 10 min. Amplification was confirmed by agarose gel electrophoresis.

**TGGE.** Samples were analyzed, as previously described, using a TGGE apparatus (Diagen, Düsseldorf, Germany) (15). The temperature gradient, running time, position of the loading wells for parallel TGGE, and gel concentration were established by diagonal TGGE of each PCR product corresponding to each pair of primers. Other DNA samples were analyzed using single-stranded conformational polymorphism (SSCP) on PCR products generated with exon-specific primers based on adjacent intronic sequences. All abnormalities discovered by TGGE and SSCP screening were confirmed by direct sequencing.**

**TABLE 1. Patient population by diagnosis, mode of inheritance, and neuroendocrine studies**

<table>
<thead>
<tr>
<th>Inheritance</th>
<th>Sporadic n = 38</th>
<th>Familial n = 21</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>KAL Mutations</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>1/1 X-L</td>
<td>0</td>
</tr>
<tr>
<td>2/6 Indeterminate</td>
<td>0/1 X-L</td>
<td>0/1 Indeterminate</td>
</tr>
<tr>
<td>0/8 AD</td>
<td>0/4 AD</td>
<td></td>
</tr>
<tr>
<td>0/6 AR</td>
<td>0/7 AR</td>
<td></td>
</tr>
<tr>
<td><strong>Neuroendocrine studies</strong></td>
<td>KAL gene</td>
<td>KAL gene</td>
</tr>
<tr>
<td>NL gene Mutant</td>
<td>n = 10</td>
<td>n = 2</td>
</tr>
<tr>
<td>Low Amplitude LH</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Apulsatile LH</td>
<td>7</td>
<td>2</td>
</tr>
</tbody>
</table>

*Patients with novel mutations as described in this study (n = 3) and patients previously described by our group (n = 4) (19).
TABLE 2. Genotype-phenotype characteristics of 9 probands with Kallmann syndrome due to KAL mutations

<table>
<thead>
<tr>
<th>Exon</th>
<th>Nucleotide &amp; AA change</th>
<th>Genotype</th>
<th>PT</th>
<th>Mode of inheritance</th>
<th>Cryptorchidism</th>
<th>Microphallus</th>
<th>UL Renal agenesis</th>
<th>Testicular volume/testosterone</th>
<th>Other</th>
<th>LH Pattern</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>TGT to C GT Cys172 to Arg</td>
<td>1</td>
<td>3 Brothers affected</td>
<td>No (BL in both brothers)</td>
<td>No</td>
<td>No (+ in one brother)</td>
<td>TV = 1–2 mL</td>
<td>T = not available</td>
<td>Synkinesia</td>
<td>Apulsatile</td>
</tr>
<tr>
<td>5</td>
<td>CGA to TGA Arg191 to Stop</td>
<td>2</td>
<td>Sporadic</td>
<td>UL</td>
<td>No</td>
<td>N/A</td>
<td>TV = 1 mL</td>
<td>T = 143 ng/dl</td>
<td>N/A</td>
<td>Apulsatile</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>Sporadic</td>
<td>BL</td>
<td>Yes</td>
<td>No</td>
<td>TV = 1 mL</td>
<td>T = 48 ng/dl</td>
<td>N/A</td>
<td>Apulsatile</td>
</tr>
<tr>
<td>7</td>
<td>TAC to TAG Tyr288 to Stop</td>
<td>4</td>
<td>X-linked</td>
<td>No</td>
<td>No</td>
<td>N/A</td>
<td>TV = 1 mL</td>
<td>T = 62 ng/dl</td>
<td>High-arched palate; Color blindness</td>
<td>Apulsatile</td>
</tr>
<tr>
<td>8</td>
<td>11 Base deletion Splice at Asn100</td>
<td>5</td>
<td>X-linked</td>
<td>BL</td>
<td>No</td>
<td>N/A</td>
<td>TV = 1 mL</td>
<td>T = 95 ng/dl</td>
<td>N/A</td>
<td>Apulsatile</td>
</tr>
<tr>
<td>10</td>
<td>14 Base deletion Frameshift at Pro164</td>
<td>6</td>
<td>Sporadic</td>
<td>No</td>
<td>N/A</td>
<td>N/A</td>
<td>TV = 1 mL</td>
<td>T = not available</td>
<td>Color blindness</td>
<td>Apulsatile</td>
</tr>
<tr>
<td>10</td>
<td>CTA to TGA Arg177 to Stop</td>
<td>7</td>
<td>X-linked</td>
<td>UL</td>
<td>No</td>
<td>N/A</td>
<td>TV = 8 mL (R)</td>
<td>T = 10 ng/dl</td>
<td>Apulsatile</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Sporadic</td>
<td>8</td>
<td>UL</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>TV = 1 mL</td>
<td>T = 1 ng/dl</td>
<td>Apulsatile</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>1 Base deletion (C) Frameshift at Leu680</td>
<td>9</td>
<td>2 Brothers affected</td>
<td>No</td>
<td>No</td>
<td>N/A</td>
<td>TV = 2 mL</td>
<td>T = 95 ng/dl</td>
<td>Synkinesia</td>
<td>Apulsatile</td>
</tr>
</tbody>
</table>

a Patients previously described (19). UL/BL, Uni/bilateral; N/A, not accessed; TV, testicular volume; T, testosterone.
Conversely, kindreds with anosmic women who gave birth to sons with KS did not harbor KAL mutations (n = 3).

**Mutations, polymorphisms, and genotype/phenotype correlations**

The four novel mutations in the KAL gene detected in the present study (C172R, R191X, R457X, and delC@L600) are depicted in Table 2 along with their phenotype, in addition to mutations published previously by our group (19). All were found in patients in whom hypogonadotropic hypogonadism was accompanied by anosmia (Fig. 1). One mutation corresponds to a T-to-C substitution at nucleotide 664 (TGT to CGT) in exon 4 (the WAP region), changing cysteine into arginine at codon 172 (C172R). This mutation was found in each of three brothers with KS. The second novel mutation consists of a C-to-T substitution at nucleotide 721 in exon 5 (CGA to TGA) that changes an arginine into a stop at codon 191 (R191X) (fibronectin III repeat domain). This mutation was detected in two cases of sporadic KS. In the third novel mutation, a T substitutes for an A at nucleotide 1519, changing codon 457 from an arginine to a stop codon (CGA to TGA, R457X) (fibronectin III repeat domain). This mutation was detected in two unrelated patients, one with sporadic KS and the other with X-linked KS. The final mutation consists of a single base deletion of an A at nucleotide 1951, causing a frameshift in exon 12, and a stop codon (TAA) to be introduced at position 618 (exon 13). This mutation was identified in two brothers.

We also found two previously described polymorphisms (20). The first is a substitution of an A for a G at codon 534, changing valine into isoleucine. This change was identified in only 67% of our population, a somewhat higher percentage, compared with previous reports of 48% in normals (20). The second polymorphism is a T-to-C substitution in exon 12 at codon 611 that was detected in 32% of our patients. This conservative base pair change (isoleucine) has been previously identified in 24% of controls (20). Polymorphisms previously described in exons 2, 13, and 14 (19, 20) were not detected in our population.

The clinical characteristics of the patients with KAL mutations are described in Table 2. Individuals with the same mutation presented with different phenotypes, even within the same family. For example, two unrelated KS individuals presented with the same mutation in exon 5 (R191X) but different phenotypes. One patient was born with microphallicus and bilateral cryptorchidism (patient 3), whereas the other individual had only right cryptorchidism (patient 2). Lack of concordance between genotype and phenotype was also observed within families. In the KS family with three affected brothers all harboring the C172R mutation, synkinesis was present in all three, bilateral cryptorchidism was present in two, but only one brother had unilateral renal aplasia.

**Neuroendocrine patterns of secretion**

Thirty-seven individuals who underwent mutation analysis, as described here and in a previous report by our group (19), underwent frequent blood sampling (21 KS and 16 IHH). Seventy-three percent (n = 27) exhibited an apulsatile pattern of LH secretion (#1 LH peak in 24 h), and 27% (n = 10) presented with low amplitude pulses (three or more pulses in 24 h) (Table 1). All individuals bearing KAL mutations that were studied with frequent blood sampling (n = 8) demonstrated an apulsatile pattern of LH secretion. Three of these eight individuals were members of the same family. Figure 2 shows the data for three representative individuals. A shows a proband (patient 5 in Table 1) from an X-linked pedigree with a documented KAL mutation (splice mutation at Asn400) and with an apulsatile pattern of LH secretion during a q 10-min blood sampling study. B shows a proband from a familial KS pedigree, albeit indeterminate in its mode of inheritance, with no KAL coding sequence mutation. The pattern of LH secretion for this proband is again apulsatile but with some possible evidence of LH secretion. C shows a male proband from an autosomal dominant pedigree with no detectable KAL coding sequence mutation. However, the neuroendocrine profile of this proband demonstrates not only measurable LH levels but also two LH pulses.
Discussion

This study supports and quantifies the importance of autosomal genes in producing KS. Whereas much attention has been focused upon the discovery, analysis, and clinical features of cases with the X-linked form of KS, this study makes it clear that most cases of familial (and presumably sporadic) KS are either autosomal dominant or recessive in their etiology. It is also clear that there are striking phenotypic differences between those with mutations in the \textit{KAL} gene and those with autosomal modes of inheritance.

Of the 101 probands, 59 of which were anosmic and 21 of 59 which were familial, only a minority of the kindreds had a pedigree consistent with X-linkage. However, it was theoretically possible that KS caused by \textit{KAL} mutations might include a more variable phenotype; hence, a comprehensive mutational screening approach was taken to address this issue. However, this study does not support the expansion of the X-linked KS phenotype to include patients with IHH with normal olfaction, families in which individuals with normal and impaired sense of smell coexist, nor a female carrier state in which anosmia/hyposmia or reproductive abnormalities occur. However, X-linked cases with confirmed mutations in \textit{KAL} did exhibit a spectrum of phenotypes both within and across families with identical genotypes. In addition, they all demonstrated a complete absence of LH pulsations during frequent blood sampling. Given the evidence of failure of GnRH neurons to migrate beyond the cribriform plate in a single case of KS in whom a \textit{KAL} gene defect was confirmed (4), this complete absence of GnRH-induced LH pulses fits with the failure of these neurons to arrive in the medial basal hypothalamus and thus participate in the induction of pulsatile LH secretion. Conversely, the presence of low-amplitude LH pulses in autosomal cases documents some degree of GnRH secretion. Thus, the neuroendocrine profiles should be considered as an additional intermediary phenotype in this complex disorder. For the moment, the genes underlying the autosomal cases of KS remain to be discovered.

Fig. 2. LH secretion in KS. A, B, and C, Baseline secretory pattern of LH in three individuals with KS (arrow) assessed at 10-min intervals; \textit{shaded area}, lower limit of range of serum LH concentrations defined in normal men (35); T, serum testosterone measured in pooled samples constituted from equal aliquots of the frequent blood samples; TV, testicular volumes recorded using a Prader orchidometer; \textit{inverted triangles}, LH pulses.
Our findings demonstrate that the frequency of mutations in the coding sequence of the KAL gene is low in both familial and sporadic Kallmann patients. Whereas coding sequence mutations in the KAL gene have previously been reported in as many as 50–60% of familial cases (5, 6, 20), these investigators have largely focused upon X-linked kindreds. However, the mode of inheritance of pedigrees included in these prior studies has occasionally been ambiguous (5). In addition, at least three families with apparent X-linked inheritance have been reported with no KAL coding sequence mutations, suggesting that mutations may occur in noncoding regions of the KAL gene (5, 6). However, polymorphic allele patterns of these pedigrees were not examined to determine that these kindreds were, in fact, linked to the KAL locus. Therefore, the formal possibilities remain that another X-linked gene causes KS or that these families were not truly X-linked. Nevertheless, our finding of only three KAL mutations in the entire cohort of anosmic familial cases (n = 21), combined with other pedigree features, certainly demonstrates that the majority of familial KS is attributable to autosomal (and not X-linked) genes.

Since the KAL gene escapes X inactivation, obligate carrier mothers of KS patients harbor both normal and mutated alleles. The presumption that these women have a decrease in anosmin levels raises the possibility of a partial phenotype in either reproductive or olfactory function. Partial expression of X-linked diseases in female carriers has been described in numerous conditions (21–24). In fact, our group has described female carriers in another X-linked form of hypogonadotropic hypogonadism (congenital adrenal hypoplasia) with a reproductive phenotype (25). Previous studies have never clearly addressed whether KAL mutations may be present in normosomic patients. Kirk et al. (26) performed olfactory testing for KS in eight female carriers who were identified on the basis of the birth of an affected son. No genetic data were available on these patients except for a single family that was known to harbor a 7Mb interstitial deletion on Xp. As a group, the female KS carriers had a lower median threshold for detection of all odorant classes, compared with the control groups. However, considerable overlap of the KS carriers with unaffected relatives was apparent. Nonetheless, these authors concluded that obligate female carriers were hyposmic, but with considerable overlap with normals, making olfactory testing unsatisfactory for detection of carrier status.

Therefore, this study sought to examine whether anosmic women with normal reproductive function might actually have KAL mutations. We evaluated three families in which anosmic women gave birth to sons with KS, and none had any mutation in the coding sequence of the KAL gene. Conversely, three obligate female KAL mutant carriers, identified in our screening program, had no olfactory defect by history (although formal testing was not performed). The absence of documented mutations in the KAL coding sequence in women with anosmia in KS families and the apparently normal olfactory function in female carriers with documented KAL mutations provide additional evidence against isolated anosmia being a partial phenotype of KAL mutations. The occurrence of isolated anosmia (without IHH) as an alternative phenotypic manifestation of the same genetic defect within a given KS family thus seems to be a feature of the autosomal dominant cases that demonstrates variable expressivity.

In addition to examining isolated anosmia as a possible surrogate marker for KAL mutations, we also examined the possibility that normosmic IHH might be a partial phenotype for KS. However, two pedigrees in which siblings had both IHH and KS also did not exhibit a KAL mutation.

Variable expression of other phenotypic features associated with KS (i.e. synkinesia, renal agenesis, and cryptorchidism) has been well documented. However, neuroendocrine profiles of LH secretion have not been reported in detail for this condition. Previous studies have shown that the GnRH neurons originate in the olfactory placode and migrate into the brain along branches of the terminalis and vomental nerves (27). Schwanzel-Fukuda et al. (4) studied a KS fetus with a deletion from Xp 22.31 to Xpter, i.e. encompassing the entire KAL gene, and demonstrated that migration of the GnRH and olfactory neurons was arrested just below the telencephalon at the cribiform plate. No GnRH expression in cells or fibers was detected in the brain, yet dense clusters of GnRH cells and thick fascicles of GnRH fibers remained in the nose. Mutations in KAL (and by extension, anosmin) seem to cause premature failure of migration of the olfactory neurons and, consequently, of the GnRH neurons to the brain. We demonstrated that eight patients with mutations in the coding sequence of the KAL gene that were studied with 10-min blood sampling exhibited uniformly apulsatile patterns of LH secretion and, by extension, a complete absence of GnRH secretion. Though other familial cases may also demonstrate an apulsatile pattern, we identified a familial, non-X-linked case that had evidence of low-amplitude LH pulses (Fig. 1). Thus, it seems that some non-X-linked KS cases may not be attributable to abnormalities in GnRH migration, having some GnRH-induced LH signaling, albeit attenuated, present. Obviously, additional neuroendocrine studies are required to address this issue. Interestingly, however, at least by the data presented in this study, apulsatile LH secretion may be one of the more consistent phenotypic features of KS attributable to KAL mutations.

Previously described mutations in the KAL gene are distributed throughout the gene, although most point mutations cluster in the four fibronectin type III repeat (FNIII) domains (Fig. 1) (5, 6, 19, 20, 28–31). However, mutations in the cysteine-rich region were also published in KS patients presenting with synkinesia and renal malformations, the most common nonreproductive abnormalities associated with this syndrome. The C172R mutation described in the WAP-region was identified in a family with three KS brothers, all of whom presented with synkinesia in addition to their reproductive disorder. One brother in this family also had unilateral renal aplasia, as described previously for mutations in the FNIII region. Two unrelated patients with the KAL mutation, W258X, have been reported (6); one had bimanual synkinesia, left renal aplasia, and cryptorchidism whereas the other had no such phenotypic anomalies. Hardelin et al. (5) described another patient with the same W258X mutation, presenting with synkinesia and unilateral renal aplasia but no cryptorchidism. Thus, there seems to be extensive variability in the phenotypic expression of mutations.
both within and between families (2, 5, 6, 19, 20, 28, 30–34). Even though the mutations are more frequently located in the FNIII region, there do not seem to be hot spots in the KAL gene. There is also no correlation between phenotype and location of the mutation (5).

In summary, a small proportion of both familial and sporadic cases of KS carry KAL mutations. Considering just the familial cases, KAL mutations were identified only in families that were clearly X-linked or in which only brothers were affected. No mutations were found in nonmosaic IHH patients in families with both IHH and KS patients or in families with anosmic women with normal reproductive function and KS males. In our small population of genetically proven female carriers, anosmia does not seem to represent a partial phenotype. Affected individuals with KAL gene mutations have aplasitic LH secretion. Autosomal genes causing KS exist, seem to account for the majority of cases, and may have unique neuroendocrine phenotypes.

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