Cytogenetic Analysis and Detection of KAL-1 Gene Deletion With Fluorescence In Situ Hybridization (FISH) in Patients With Kallmann Syndrome

**ABSTRACT**

Kallmann syndrome (KS) is a disease clinically characterized by the association of hypogonadotropic hypogonadism and anosmia or hyposmia, for which three modes of transmission have been described: X-linked, autosomal recessive and autosomal dominant. The KAL-1 gene, responsible for the X-linked form of the disease, has been isolated and its intron-exon organization determined. In this study, two families with X-linked KS and four sporadic male patients with hypogonadotropic hypogonadism and anosmia were cytogenetically investigated with high-resolution techniques and FISH. Chromosomal analysis did not reveal any rearrangements or deletions. Deletion of the KAL-1 gene was detected by FISH in only one sporadic patient, with the typical features of KS and a high palate. Among the familial cases renal abnormalities and pes cavus deformity were observed. (Arq Bras Endocrinol Metab 2001;45/6:552-557)

**Keywords:** Kallmann syndrome; Deletion KAL-1; FISH; Renal abnormalities.

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**KALLMANN SYNDROME** is a disease clinically characterized by the association of hypogonadotropic hypogonadism and anosmia or hyposmia. The hypogonadism is due to insufficient release of gonadotropin releasing hormone (GnRH) from the hypothalamus (1), while anosmia has been related to agenesis of the olfactory bulbs (2). Since this disorder was described, patients with hypogonadotropic hypogonadism have classically been divided into those with anosmia (KS) and those with normal olfaction [idiopathic hypogonadotropic hypogonadism (IHH)].

Olfactory testing is available to characterize sense of smell and differentiate the individuals with KS from IHH (3,4), although this differentiation may be difficult because of variability in expression of anosmia in KS (5). In addition, magnetic resonance imaging may also be helpful due...
to the detection of olfactory anatomic abnormalities in most, but not all, KS patients (6).

Though mainly sporadic, familial cases were first reported by Kallmann in 1944 (7). Subsequent segregation analyses revealed X chromosome-linked, autosomal recessive and autosomal dominant modes of transmission indicating genetic heterogeneity (8-10). The incidence of KS has been estimated as one in 10,000 males and one in 50,000 females (11). The 5 to 7 fold excess of affected males versus females originally suggested that the X-linked mode (MIM 308700) of inheritance was the most frequent (12). However, it has recently been demonstrated that the X-linked form of the disease accounts for the minority of patients and that most affected subjects are due to mutations in autosomal genes (13,14).

The KAL-1 gene for X-linked KS was isolated by two independent groups, using approaches typically employed in positional cloning. Characterization of the KAL-1 gene structure revealed the presence of 14 exons spanning approximately 210kb on Xp22.3 and shown to encode a protein sharing homology with molecules involved in neuronal migration and axonal pathfinding (15,16). The gene escapes X-inactivation and has a closely related homologue on the Y chromosome, which is nonfunctional (17,18). The finding of mutations in patients affected by KS has demonstrated that the KAL-1 gene is responsible for the X-linked form of the disease (19-21). Furthermore, some of the additional clinical anomalies occasionally observed in KS, such as mirror movements (21-23), pes cavus deformity (22), unilateral renal aplasia (24), and high-arched palate (25) could be ascribed to the altered KAL-1 gene.

KS rarely occurs as the result of a deletion involving only the KAL-1 gene (19,21). Deletions of this gene are most frequently observed in males with a contiguous gene syndrome, including the loss of genes for ichthyosis, chondrodysplasia punctata, mental retardation and short stature in the distal short arm of the human X chromosome (26,27).

The development of a fluorescence in situ hybridization (FISH) probe for the KAL-1 gene and its use in complementary routine diagnostic procedures can contribute to the etiologic investigation of hypogonadotropic hypogonadism. In the present study, high-resolution chromosome technique and FISH analysis were used to evaluate the cytogenetic status of four males from two families with the X-linked form and four sporadic cases of KS.

**SUBJECTS AND METHODS**

Four sporadic cases of KS and four patients derived from two families were included in this study. In the first family (Figure 1A) two members were affected by KS and the X-linked mode of transmission was determined according to the following criteria: presence of asymptomatic females carriers, presence of another affected male in the maternal family or among male siblings, absence of affected females, and absence of male to male transmission. In the second family (Figure 1B), two males with hypogonadotropic hypogonadism were

![Figure 1. Heredograms of two families with X-linked KS.](image)
investigated because of the presence of kidney abnormalities, which are typically observed in patients with KAL-1 mutations. Hyposmia was referred for one of the brothers (KS-3).

Diagnostic criteria for KS were clinical signs and symptoms of hypogonadism, serum testosterone levels in the hypogonadal range (0.07-1.94 ng/mL), gonadotropin levels below the normal adult male range (LH: 0.01-2.0 mUI/mL / FSH: 0.01-1.8 mUI/mL), normal baseline levels of other anterior pituitary hormones, and normal radiological imaging of the hypothalamic-pituitary region. Evaluation of olfactory function was not performed and the cases with anosmia/hyposmia were found on direct inquiry. Patients’ clinical features are summarized in Table 1. This study was approved by the Ethics Committee, Faculdade de Ciências Médicas, Universidade Estadual de Campinas (UNICAMP).

Cytogenetic investigation: Peripheral blood lymphocytes (0.5 mL) were cultured for 72 hours at 37°C in 10 mL of RPMI-1640 (Nutricell) medium supplemented with 20 percent fetal calf serum (Nutricell) and 0.2 mL of phytohemagglutinin P (Difco). After 70 hours of incubation, freshly dissolved ethidium bromide 10 µg/mL (Sigma) was added to the cultures in order to obtain prometaphase chromosomes. Colchicine (0.05 µg/mL - Sigma) was added in the last half-hour of incubation. The cells were spun down and suspended in 0.075M KCl for 50 min at 37°C and then fixed 3 times with 1:3 acetic acid-methanol. The cells were spread on clean slides by air-drying with flaming. G bands were obtained by mild treatment with 0.125% trypsin (Gibco) for 5-15 s, and stained for 10 min in Giemsa. A minimum of 32 cells was analyzed for each patient.

Fluorescence in situ hybridization (28) for detection of the KAL-1 gene locus was performed with Locus Specific Identifier-Kallmann/Chromosome Enumeration Probe X dual color DNA probes (LSI-Kallmann/CEPX, Vysis incorporation) following manufacturer’s protocol. Cells were considered normal if they had red and green signals in the analysis of 30 metaphase and prometaphase chromosomes. Normal control specimens were incorporated into each FISH assay.

Digital images were obtained using an Olympus BX 60 epi-fluorescence microscope equipped with a Cyto Vision™ system (Applied Imaging Corporation), for capture and image analysis.

RESULTS

In all individuals, the examination of GTG-banded prometaphase chromosomes revealed normal male karyotypes (46,XY). FISH analysis with Kallmann probe revealed absence of KAL-1 gene in the KS-5 case. Figure 2 shows the deletion detected in this patient and a normal male control. In all the other cases, both signals were detected on the X-chromosomes, like on the normal male control.

DISCUSSION

In the present study, we describe the finding of a deletion of KAL-1 gene in one patient, among 4 sporadic cases of Kallmann syndrome. This deletion was detected only by FISH since the chromosomal analysis showed a normal karyotype. The patient exhibited the typical clinical features of KS and a high palate.

Table 1. Clinical features of patients with hypogonadotropic hypogonadism with or without anosmia.

<table>
<thead>
<tr>
<th>Nº</th>
<th>Age at last visit</th>
<th>Clinical features associated with gonadotropin deficiency</th>
<th>Inheritance</th>
</tr>
</thead>
<tbody>
<tr>
<td>KS-1</td>
<td>32</td>
<td>+ + - + unilateral renal agenesis, pes cavus</td>
<td>X-linked</td>
</tr>
<tr>
<td>KS-2</td>
<td>28</td>
<td>+ + + - unilateral renal agenesis</td>
<td>X-linked</td>
</tr>
<tr>
<td>KS-3</td>
<td>35</td>
<td>-/+* + + + horseshoe kidney</td>
<td>X-linked</td>
</tr>
<tr>
<td>KS-4</td>
<td>28</td>
<td>- + + - unilateral renal agenesis mental retardation</td>
<td>X-linked</td>
</tr>
<tr>
<td>KS-5**</td>
<td>27</td>
<td>+ + + - high palate</td>
<td>sporadic</td>
</tr>
<tr>
<td>KS-6</td>
<td>24</td>
<td>+ + - pes cavus</td>
<td>sporadic</td>
</tr>
<tr>
<td>KS-7</td>
<td>47</td>
<td>+ + - ataxia, hypertelorism</td>
<td>sporadic</td>
</tr>
<tr>
<td>KS-8</td>
<td>37</td>
<td>+ + -</td>
<td>sporadic</td>
</tr>
</tbody>
</table>

A: anosmia; Mp: micropenis; Cp: cryptorchidism; Gn: gynecomastia
* hyposmia; ** patient with KAL-1 deletion detected by FISH
The families have molecular alterations in KAL-1 gene and it is of interest that unilateral renal agenesis occurs in half of all males with mutations (19). In the present X-linked KS, all individuals exhibited renal abnormalities. These and other reports corroborated the hypothesis that KAL-1 gene plays a yet undefined role in kidney development (33). Nevertheless, the mutation is not invariably associated with renal failure (34,35). In fact, the description of a large family with a high frequency of renal agenesis, either in the presence or in the absence of a KAL-1 gene mutation suggests the existence of another gene that contributes to renal agenesis (36).

In conclusion, the cytogenetic evaluation in a series of KS patients indicates that FISH can be useful for the detection of complete KAL-1 gene deletion in cases with features consistent with KS independently of its familial occurrence.

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