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Definitive Localization of X-linked Kallman Syndrome (Hypogonadotropic Hypogonadism and Anosmia) to Xp22.3: Close Linkage to the Hypervariable Repeat Sequence CRI-S232

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Summary

Kallmann syndrome is a genetically heterogeneous disease characterized by hypogonadotropic hypogonadism and anosmia. Six families in which the disorder followed an X-linked inheritance were investigated by linkage analysis. Diagnostic criteria were uniformly applied and included tests for hypogonadotropic hypogonadism and anosmia. Close linkage was found by using the hypervariable repeated sequence CRI-S232 (DXS278) previously mapped to Xp22.3. At a maximum lod score of 6.5, the recombination fraction was calculated as .03. Of 30 fully informative meioses, one recombination between the disease locus and the loci recognized by probe CRI-S232 was observed. When an independent approach is used, these results confirm the X-linked Kallmann syndrome assignment previously made by deletion mapping, and allow definitive localization of the syndrome to the Xp22.3 region. This opens the way to carrier detection and to the identification of a gene responsible for this disorder.

Introduction

It was the association between hypogonadism and colorblindness which, by his own account, constituted the starting point of Kallmann et al.'s (1944) paper "The Genetic Aspects of Primary Eunuchoidism." They described an X-linked Mendelian disorder characterized by hypogonadism and anosmia. In two of three families these traits showed association with colorblindness, a then known X-linked marker. Since then genetic heterogeneity has become a hallmark of the syndrome. Recessive, dominant, and X-linked pedigrees have been described, although most cases are sporadic (Santen and Paulsen 1973; White et al. 1983; Hermanussen and Sippell 1985).

Clinically, the diagnosis of Kallmann syndrome is less ambiguous. The endocrinological features include hypogonadism due to deficiency of hypothalamic gonadotropin-releasing hormone (GnRH). Anosmia has been shown to be due to agenesis or hypoplasia of the olfactory bulbs (Weidenreich 1914; Klingmüller et al. 1987). Cryptorchidism is described as an additional feature in many cases. In X-linked families, synkinesia (Kallmann et al. 1944) and, less frequently, unilateral kidney aplasia are observed (Wegencke et al. 1975). Substitution therapy is necessary to induce secondary sex characteristics. Timely treatment with GnRH and/or gonadotropins leads to fertility in the majority of cases (Burris et al. 1988). Early diagnosis of this disorder is therefore important.

In previous studies we have tentatively assigned the X-linked KAL locus to the Xp22.3 region by the study of patients with interstitial and terminal deletions (Andria et al. 1984; Ballabio et al. 1986, 1989; Petit et al. 1990). In the present paper we test our previous hypothesis by an independent approach, using linkage analysis in families with isolated X-linked Kallmann syndrome.

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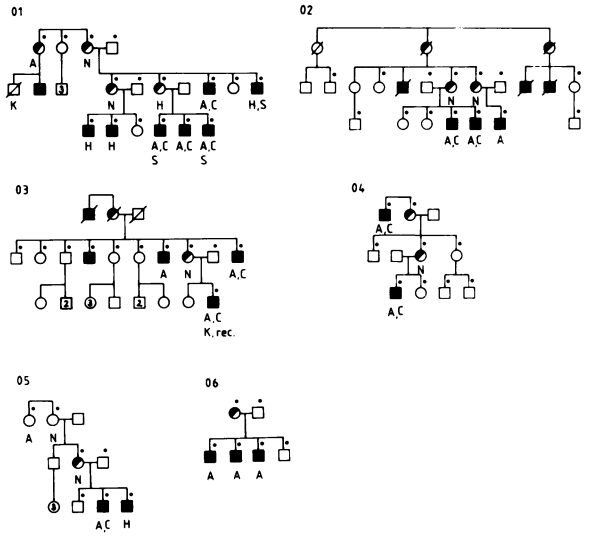

Figure 1 Six pedigrees with X-linked Kallmann syndrome. Affected males and obligate carrier females are indicated. Prepubertal males are excluded from the pedigrees. Clinical features are shown below the corresponding pedigree symbols: A = anosmia; C = cryptorchidism; K = unilateral kidney aplasia; H = hyposmia; N = normal sense of smell; S = synkinesia. Individuals from whom blood samples were available and on whom DNA analysis was performed are marked with a dot. The recombination was observed in pedigree 03 (rec.).

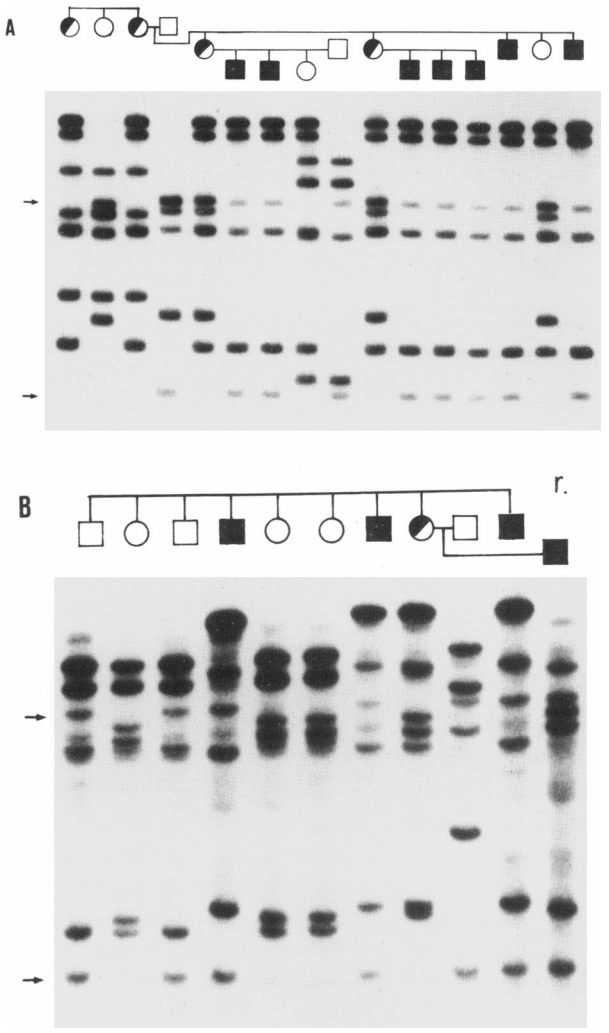

Figure 2 Southern blot analysis of pedigrees 01 (*A*) and 03 (*B*). *Eco*RI-digested DNA samples were hybridized by using pCRI-S232 as a probe. One recombination event was observed in pedigree 03 (r.). Constant Y-specific bands at 5.6 kb and 16 kb are marked with an arrow.

Material and Methods

Family Studies

Pedigree data were obtained from six Caucasian families (fig. 1). The multiallelic nature of the probes used ensured that incorrect assumptions concerning paternity were virtually excluded (fig. 2). In four families, affected individuals in at least 2 generations were connected through unaffected females. In one family (pedigree 05), X-linked inheritance was assumed on the basis of congenital anosmia in a maternal aunt of two affected brothers. In pedigree 06, affected males could only be demonstrated in 1 generation. Diagnosis of affected males was based on (1) clinical examination, (2) hormone tests (basal and human chorionic gonadotropin–stimulated testosterone levels, stimulation of plasma gonadotropins by single dose and/or pulsatile GnRH application) (Partsch et al. 1985), and (3) the semiquantitative evaluation of smell by using at least seven pure olfactory substances and various combination substances for olfactory and trigeminal or gustatory stimulation, according to the generally accepted criteria (Rosen et al. 1979). To exclude intracranial lesions sella X-ray and computer tomography were performed. Males who underwent spontaneous pubertal development are listed as unaffected in figure 1. Prepubertal males were excluded from the study. The clinical features in pedigrees 03 and 04 have been published elsewhere (Evain-Brion et al. 1982; Hermanussen and Sippell 1985).

RFLP Analysis

DNA was prepared from peripheral blood leukocytes of blood samples from 63 family members. Aliquots

Table 1

Molecular Probes Used in the Present Study

Probe	Locus	RFLP	Fragments (kb)	Frequency	No. of Recombinations Observed/ No. of Fully Informative Meioses	Reference
M1A.....	DXS31	*Xmn*I	3.0/3.4	.81/.1[a]	2/7	Wrogemann et al. 1986
BW14.....	STS	*Xmn*I	4.7/6.8	...	0/5	Wirth and Gal 1989
CRI-S232.....	DXS278	*Eco*RI	...[a]	PIC > .5	1/30	Knowlton et al. 1988
dic56	DXS143	*Bcl*I	7.4/8.9	.56/.44	0/5	Middlesworth et al. 1985
TAK10A	DXS89	*Msp*I	6.4/4.7	.81/.19	5/5	Alitalo et al. 1988

[a] Multiple alleles.

of 5 μg DNA were digested with 10 U of appropriate restriction enzymes and were separated electrophoretically in 0.7% agarose gels. Nylon filters (Amersham) were used for alkaline blotting. Hybridization was carried out at 64°C with [^{32}P]-labeled probes by using a protocol described by Church and Gilbert (1984). Probes used are listed in table 1.

Linkage Analysis

Version 5.04 of LINKAGE was used for linkage analysis (Lathrop and Lalouel 1984). Affection status of males was coded as shown in figure 1. All females were coded as status unknown. Lod scores were calculated at recombination intervals of .01, with as many as five different alleles segregating in each family. Kallmann syndrome was assumed to be an X-recessive, fully penetrant trait with a mutation rate of 10^{-6}. The following allele frequencies were used for the calculations: KAL, .0001 for the mutant allele; CRI-S232, .2 for each allele.

Probes

Polymorphic DNA sequences used in the study are summarized in table 1. Probe CRI-S232 is a hypervariable element detecting multiple bands on human DNA with various restriction enzymes. The hybridization pattern is characterized by a subset of highly polymorphic bands mapped to Xp22.3 and by a subset of constant bands on Yq11 (Knowlton et al. 1988).

Results

Clinical Features

Twenty-one males in six families included in the linkage study showed symptoms characteristic of Kallmann syndrome (fig. 1). Hypogonadotropic hypogonadism is clearly inherited as an X-recessive trait in all pedigrees except for pedigrees 05 and 06, in which alternative modes of inheritance cannot be excluded. Therefore, two different combined lod scores are provided (table 2).

Table 2

Lod Scores for Two-Point Linkage: KAL vs. DXS278

Pedigree (n[a])	No. of Recombinations	Lod Score at θ of .01	.02	.03	.04	.05	.15	Z_{max}	θ_{max}
01 (16).....	0	3.80	3.74	3.68	3.62	3.55	2.89	3.86	.00
02 (7).....	0	1.33	1.31	1.28	1.26	1.24	1.00	1.35	.00
03 (11).....	1	.10	.37	.52	.61	.67	.80	.81	.12
04 (9).....	0	1.04	1.02	1.00	.98	.96	.75	1.06	.00
05 (14).....	0	.88	.87	.86	.84	.83	.68	.90	.00
06 (6).....	0	.89	.87	.85	.83	.81	.62	.90	.00
Overall:									
01–06 (63).....	1	8.04	8.18	8.19	8.14	8.06	6.74	8.19	.03
01–04 (43).....	1	6.27	6.44	6.48	6.47	6.42	5.44	6.48	.03

[a] Number of family members available for linkage study.

Definitive evidence of hypogonadotropic hypogonadism by recording basal and stimulated gonadotropin levels and basal testosterone was obtained in all cases but one (fig. 1, pedigree 01, individual II-2). Reduced sense of smell is a constant finding and can present either as hyposmia or as anosmia. There is evidence that the degree of olfactory dysfunction is correlated with that of hypogonadism (B. Heye, unpublished data). Three cases of synkinesia were observed in pedigree 01 (fig. 1). Unilateral kidney agenesis could be demonstrated in two individuals, one in pedigree 01 and one in pedigree 03. No individual showed signs of ichthyosis.

Twelve transmitting females could be identified on the basis of the pedigrees. They did not display any obvious endocrinological changes. Fertility was normal, with no increase in the rate of either spontaneous abortion or stillbirth observed. No measurements of gonadotropin levels were made. These would be difficult to assess against the background of normal variation in females of different age. Delayed puberty was not a feature observed among the sample of obligate carriers. Sense of smell varies among carrier females clinically investigated. Hyposmia was recorded in one female, and anosmia was observed in two females.

Two-Point Linkage Analysis

Of the five probes used in the linkage study, only the highly polymorphic one was informative to an extent which resulted in high lod scores (table 1). No recombination was observed with probes dic56 and BW14 in five informative meioses. No deletions were observed in any of the affected individuals when the probes listed in table 1 were used.

Probe CRI-S232 turned out to be informative in every meiosis scored. The probe recognizes a number of homologous regions on Xp22.3 and Yq11.2, resulting in a complex but easily interpretable band pattern (Knowlton et al. 1988). For linkage analysis, probe CRI-S232 has been considered as a single locus, since recombinations among the various CRI-S232 hybridizing fragments have not been observed in the families we studied. When pedigrees 05 and 06 are excluded, the recombination fraction (θ) maximum-likelihood estimate (θ_{max}) between KAL and DXS278 was .03 with a maximum lod score (Z_{max}) of 6.5; the confidence limits (θ at $Z_{max} - 1$) were .001 and .15, respectively (table 2). The combined lod score for all six families investigated exceeds 8. In one affected individual (fig. 2*B*, pedigree 03), a recombination was observed between CRI-S232 and the disease locus. This recombination event was uninformative for the flanking markers dic56 and BW14.

Discussion

Clinical phenotypes in X-linked Kallmann syndrome are variable. They can range from severe and therapy-resistant gonadal dysplasia to cases which are difficult to distinguish from delayed puberty (Spratt et al. 1987). For many genetic disorders, the potential for diagnosis and the potential for therapy are far from equal; however, in Kallmann syndrome effective therapy is available, and so early diagnosis is particularly important.

We have previously described the association of X-linked Kallmann syndrome with X-linked ichthyosis, due to steroid sulfatase deficiency, in several affected members from two unrelated families (Andria et al. 1984; Ballabio et al. 1986). This observation led us to hypothesize the presence of a contiguous gene deletion involving both the STS gene, previously localized to Xp22.3, and the KAL gene. The hypothesis was further supported by the observation of similar cases (Sunohara et al. 1986; Bick et al. 1989) and by the demonstration of an interstitial deletion, involving the entire STS gene and flanking loci, in our original cases (Ballabio et al. 1987, 1989). However, families with isolated X-linked Kallmann syndrome were still needed in order to assign KAL to Xp22.3, since it could not be excluded that the features of the disorder in the deletion patients were due to pleiotropic effects or to complex interaction of other genes involved in the deletion, as suggested by Schnur et al. (1989). In a single male patient with a syndrome characterized by glycerol kinase deficiency, adrenal hypoplasia, and hypogonadism, Goonewardena et al. (1989) demonstrated an interstitial deletion in Xp close to the DMD locus. The clinical features of the hypogonadism in this case were clearly distinct from those seen in Kallmann syndrome, and therefore the respective locus is unlikely to be identical to that for Kallmann syndrome.

In the present linkage study we have reported definitive evidence for the localization of an X-linked KAL locus to Xp22.3. The most informative marker used in the analysis was pCRI-S232. Recent studies have demonstrated, using different approaches, that pCRI-S232 recognizes multiple loci in both Xp22.3 (Johnson et al. 1989; Schnur et al. 1989; Petit et al. 1990; Ross et al. 1990; Ballabio et al., in press) and Yq11.

In five families with X-linked Kallmann syndrome, two X-specific polymorphic loci, detected by pCRI-S232, were cosegregating with the disorder in every meiosis scored. In another family (pedigree 03), the two loci were cosegregating in six of seven meioses, with one individual being a recombinant between the dis-

ease locus and both the marker loci. We can conclude that no evidence exists for locus heterogeneity in the X-linked Kallmann syndrome families studied. Internal recombination between the two CRI-S232 hybridizing loci, previously described in two meioses (Knowlton et al. 1988), was not observed in the present study.

A recently refined deletion map of the Xp22.3 region places KAL in a specific deletion interval proximal to STS (Ballabio et al. 1989). A long-range map of this region pinpoints possible gene sites by identifying a number of CpG islands (Petit et al. 1990; Ross et al. 1990). None of the probes used in linkage analysis was deleted. We conclude that point mutations or small rearrangements are responsible for the disease in the families studied. Analysis of the recombination event places KAL outside the region defined by pCRI-S232. Therefore, the islands mapping just proximal to the STS locus are promising sites to search for the KAL gene. pCRI-S232 constitutes a new starting point for any attempts by chromosome walks toward the cloning of the KAL gene.

Recent studies have shed some light on the possible pathogenetic mechanism involved in Kallmann syndrome. It is now known that both the olfactory nerve and the GnRH-producing cells of the hypothalamus arise from the olfactory placode and that, during development, olfactory nerve axons induce the formation of the olfactory bulbs and tracts, while GnRH immunoreactive cells migrate to the hypothalamus (Schwanzel-Fukuda and Pfaff 1989). In a recent study, a defect in neuronal migration of cells and processes from the olfactory placode was demonstrated in a fetus who was prenatally diagnosed as having a terminal deletion of the short arm of the X chromosome and whose brother, affected by the same anomaly, displayed short stature, chondrodysplasia punctata, steroid sulfatase deficiency, and Kallmann syndrome (Schwanzel-Fukuda et al. 1989). These findings suggest that Kallman syndrome may represent a developmental defect related to cell migration.

On the basis of our results, pCRI-S232 can now be used, in a very efficient way, as a tool for diagnosing X-linked Kallmann syndrome, greatly facilitating early diagnosis and opening the way for carrier detection in this disorder. Although feasible, prenatal diagnosis may not be a frequent choice in this disorder, given the increasing success rate of hormone therapy. The unraveling of the molecular etiology of this disorder might increase therapeutic advances even further.

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