Kallmann syndrome in a boy with a t(1;10) translocation detected by reverse chromosome painting

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Abstract
Prometaphase chromosomes from a 16 year old boy with hypogonadotrophic hypogonadism and anosmia (Kallmann syndrome) showed a tiny chromosome fragment attached to the long arm of one chromosome 1 without a visible reciprocal translocation chromosome. Chromosome painting with libraries from chromosomes 1 and X excluded a t(X;1) translocation, but failed to detect a second translocation chromosome. Through reverse chromosome painting, an unbalanced der(1), t(1;10) (q44;q26) translocation could be detected. This is the third case of Kallmann syndrome with a de novo rearrangement between two autosomes. The distal long arm of chromosome 1 may contain a candidate locus for a gene, mutations of which may cause the Kallmann phenotype; a 10q location seems less likely.

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Carpal and phalangeal bone ages were 15 years at a chronological age of 15 years 8 months and predicted adult height was 1·87 m (mean adult height 1·86 m, SD 6 cm)\(^2\).

At the age of 18 years, he was operated on for cryptorchidism. The testes were described as very small (1–2 ml).

Smelling tests disclosed anosmia.

Psychological testing at the age of 20 years using the DCS, HAWIE, and Bruton tests and Rorschach tables indicated a total IQ of 72 (performance 65, verbal 78). However, in another IQ test, the SPM test which is considered independent of cultural background, he achieved an IQ of 98. Considering both tests, his IQ was judged to be between 80 and 90. Visual memory was defective as was concentration ability. Only marginal contact could be established between the patient and examiner during a four hour testing period.

**ENDOCRINOLOGICAL INVESTIGATIONS**

A GnRH test (25 µg IV) performed at the age of 15 years 7 months showed very low basal LH and FSH (both <0.5 U/l) and only a minimal, insignificant response to GnRH (maximum LH after 30 minutes 1·2 U/l; maximum FSH after 60 minutes 1·6 U/l). Plasma testosterone was prepubertal (1·69 nmol/l). Basal cortisol was normal (490 nmol/l) and responded normally to ACTH (760 nmol/l). These results confirmed the presence of hypogonadotrophic hypogonadism. Subsequently, the patient was given a substitution therapy with a long acting testosterone ester (Testoviron Depot, 250 mg per month IM).

**RADIOLICAL EXAMINATION**

On abdominal ultrasonography, small testicular-like structures were seen in the inguinal canals.

Lateral skull films disclosed a normal configuration of the sella. Magnetic resonance imaging of the brain showed normal findings including a normal hypophysis, but hypoplasia of the right and aplasia of the left olfactory bulb.
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**Figure 4** Reverse chromosome painting shows the origin of the additional chromosome segment on one homologue of chromosome 1. DOP-PCR paint probes derived from floto sorted chromosome labelled by biotin and detected by avidin-FITC were hybridised to various metaphases. Chromosomes were counterstained with DAPI/prodipidum iodide and examined under fluorescence using an MBC 600 confocal scanning microscope. (A) Chromosome 1 paint from patient's normal chromosome 1 hybridised to a normal male metaphase. Only chromosomes 1 show signal. (B) Normal chromosome 1 paint hybridised to patient's metaphase. Signal showed on both normal and derivative chromosomes 1 except for tip of the long arm of derivative chromosome (arrow). The lack of signal on chromosome 1 indicates absence of reciprocal exchange between chromosomes 1 and 10. (C) Derivative chromosome 1 paint hybridised to normal male metaphase. Both chromosomes 1 painted, except for the tip of the long arm (arrow), in addition to the distal end of the long arm of both chromosomes 10 (from 10q26 to 10qter). (D) Chromosome 10 paint derived from a der(10),t(9;10) (q22;p12) hybridised to patient's metaphase. Note chromosome 10 signal on distal end of one homologue of chromosome 1 (arrow) in addition to signals on regions of chromosomes 9 and 10 (plus weak signals on contaminating chromosomes which is an artefact of this particular probe).

**CYTOGENETIC INVESTIGATIONS**

Banded prometaphase GTG and QFQ karyotypes consistently showed a small additional segment attached to the long arm of chromosome 1 (fig 2). Fluorescent in situ hybridisation (FISH) using chromosome 1 and X specific paints failed to show evidence of a balanced translocation and, in particular, excluded a t(X;1)(p22.3;q44) translocation involving the region of the X linked Kallmann locus. Chromosome suspensions were made from cell cultures from the patient, stained with Hoechst-33258 and Chromomycin A3, and analysed by dual laser flow cytometry as previously described. Fig 3 shows the flow karyotype produced by this analysis. The translocation derivative chromosome 1 sorts slightly above and to the right of the normal chromosome 1, partly because of its increased size and partly because of its increased G-C content.

Three hundred to five hundred of both the derivative chromosome 1 and the normal chromosome 1 were sorted separately by flow cytometry and used to prepare chromosome paints for reverse chromosome painting using the DOP-PCR protocol described previously. Hybridisation of the normal chromosome 1 paint to normal metaphases painted only the normal chromosomes 1 (fig 4A). When the normal chromosome 1 paint was hybridised to metaphases from the patient, all except the distal end of the long arm of the derivative chromosome 1 was painted (fig 4B). There was no evidence of hybridisation of chromosome 1 paint on either homologue of chromosome 10, which suggested that the translocation was unbalanced. When the derivative chromosome 1 paint was hybridised to normal metaphases, signal was seen along the length of both chromosomes 1 except for band 1q44, and at the distal end of the long arm of both chromosomes 10 from 10q26 to 10qter (fig 4C). This provides further evidence of an unbalanced reciprocal translocation leading to a small deletion at 1q44 and a duplication of 10q26-qter. A chromosome 10 paint was used to confirm that segment 10q26-qter had been translocated to 1q44. As chromosome 10 cannot be sorted separately from other members of the chromosome 9 to 12 group (fig 3), a chromosome 10 derivative containing 10q26-qter was used...
as a paint probe from another cell line containing a balanced t(9;10) (q22;p12). Fig 4D shows the hybridisation of this paint probe to the patient's chromosomes; 10p12-qter and 9q22-qter regions of the homologues of both chromosomes are painted in addition to the tip of the long arm of the chromosome 1 derivative. These results indicate that the patient has an unbalanced 46,XY,-1,+der(1)t(1;10) (q44;q26) karyotype. The karyotypes of both parents are normal.

MOLECULAR INVESTIGATIONS
By Southern blot analysis with probes within the KALX gene, no shift of bands could be detected. Thus, obvious mutations in the KALX gene could be excluded (A Ballabio, personal communication).

Discussion
Kallmann syndrome (hypogonadotropic hypogonadism with anosmia) is known to be genetically heterogeneous. Mutations in an X linked gene account for an as yet unknown proportion of male patients. This gene, KALX, has recently been cloned, and mutations can be analysed. Females heterozygous for the X linked Kallmann syndrome may show minor manifestations of the disease including reduced fertility or hyposmia. Deletion of this gene, together with two genes mapping more distally on Xp, have been observed in males with ichthyosis owing to STS deficiency, chondrodysplasia punctata, mental retardation, and Kallmann syndrome.

On the other hand, at least one, but probably more autosomal genes exist, mutations of which may cause the Kallmann phenotype. Male to male transmission of the condition has been repeatedly observed. Variable expressivity is a remarkable feature in the dominant form, with some male or female carriers manifesting either only anosmia or only hypogonadism, and others showing both components of the condition. In addition, a recessive form may also exist although recessive pedigrees could also be explained by a dominant gene with incomplete penetrance.

Gene mapping through familial or de novo balanced rearrangements has proven to be a potent method both for X linked genes (especially in affected females) and for autosomal dominant genes. For autosomal recessive genes, the method will very rarely be successful; affected patients would need to have, by chance, both a disruption of one allele through a chromosome rearrangement and a mutation at the other allele.

The observation of a de novo unbalanced rearrangement between two autosomes in a patient with Kallmann syndrome could be either a chance association or, more likely, because of deletion or disruption of a gene with dominant transmission causing the condition. Two such patients have been described previously. Best et al reported a balanced de novo translocation, 46,XYt(7;12)(q22;q24), in a patient with Kallmann syndrome, and Casamassima et al reported a patient with a complex chromosomal rearrangement, 46,XYt(3;9) (9;12) (q13/2;q21.2)p13/q15. Both patients of these chromosomal rearrangements have the characteristic phenotype of Kallmann syndrome and neither showed atypical manifestations. Breakpoints in chromosome 12 were obviously at different sites on the long arm in the two patients although an additional more complex rearrangement including a minor paracentric inversion could probably not be fully excluded. The present case has two new breakpoints with an unbalanced der(1)t(1;10) translocation: 46, XYt-1,+der(1)t(1;10) (q44;q26). Thus, there are, so far, six candidate chromosomes with seven locations for a probably dominant form of Kallmann syndrome. In the two previous papers, an X linked form of KS and a chance association with an independent translocation cannot be fully excluded, since analysis for mutation in KALX was not reported, while in the patient of the present report, obvious mutations in the X linked gene could be excluded.

Furthermore, the present case is the only one with an unbalanced rearrangement, leading to disruption of the q segment and duplication of terminal 10q which makes the chromosome 1 localisation (through deletion rather than through disruption or duplication) of a Kallmann gene more likely. It is interesting to note that, despite the microscopically visible deletion, mental retardation is borderline to mild in the patient. This case illustrates the power of reverse chromosome painting to characterise complex de novo unbalanced chromosome rearrangements, as conventional cytogenetics was unable to identify the origin of the additional chromosome material on the abnormal chromosome 1.

Linkage studies in large pedigrees with autosomal dominant KS should first consider the possibility that a "Kallmann gene" could map to one breakpoint involved in the rearrangements in the three cases. It is possible that multiple "Kallmann loci" exist and that two or all three translocation cases are the result of disruption of different genes. The high probability of genetic heterogeneity should also be taken into consideration if more than one pedigree is used for linkage studies.

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