Sex, nose and genotype

A candidate for the gene that is deleted in Kallmann Syndrome has been identified and encodes a protein that may be of importance in the regulation of neuronal migration.

Cell migration is a fundamental process in the morphogenesis of all tissues. It plays an especially important role in neural development, where precisely timed waves of cell migration result in the formation of the functionally specialized laminae and nuclei of the central nervous system and the ganglia of the peripheral nervous system. The luteinizing hormone-releasing hormone (LHRH) system of the brain is a particularly dramatic example of the importance of cell migration in nervous system development. LHRH-expressing neurons have recently been shown to originate in a part of the developing olfactory epithelium of the nasal cavity (the vomeronasal organ), and to migrate during fetal life from their peripheral site of origin to their final destinations in two nearby regions of the brain: the septal preoptic area and the hypothalamus [1,2] (Fig. 1). LHRH, a hypothalamic peptide which stimulates secretion of gonadotropic hormones from the anterior pituitary, is crucial for proper development and function of the male gonad.

Patients suffering from the inherited disease, Kallmann Syndrome, have two distinct defects. In the X-linked form of Kallmann Syndrome, the hypogonadism that characterizes males with this syndrome seems to be the result of gonadotropin deficiency, secondary to inadequate or incorrectly regulated secretion of LHRH. This in turn has been suggested to be due to a failure of LHRH-producing neurons to migrate to their hypothalamic destination during fetal development [3]. The other major defect associated with Kallmann Syndrome is anosmia, or lack of the sense of smell [4]. Odor sensation is transmitted to the brain by sensory neurons called olfactory receptor neurons, and, like the LHRH neurons of the hypothalamus, these neurons are produced by the olfactory epithelium of the nasal cavity. Like LHRH neurons, olfactory receptor neurons also penetrate the brain, but in this case their cell bodies remain in their peripheral location, while their axons enter the brain to synapse upon neurons of the olfactory bulb. The anosmia of Kallmann Syndrome is associated with agenesis of the olfactory bulbs of the brain, and experimental embryologists have shown that proper development of the olfactory bulbs requires innervation by olfactory receptor neurons [5]. Thus, a failure of olfactory receptor neuron axons to penetrate the brain could potentially account for the lack of olfactory bulbs in Kallmann Syndrome patients [3].

The X-linked form of Kallmann Syndrome has been mapped to the terminal part of the short arm of the X chromosome. In one study, DNA was analysed from individuals with contiguous gene syndromes (that is, complex genetic syndromes caused by deletions of contiguous genes) resulting from deletions in Xp22.3 [6]. The Kallmann Syndrome critical interval (*KAL* interval) was assigned to the region between the breakpoint of an X/Y translocation in a patient affected by Kallmann Syndrome and four other disorders mapping to Xp22.3 (patient AM), and the breakpoint of an X/Y translocation in a patient unaffected by Kallmann but displaying the other disorders. A subsequent study

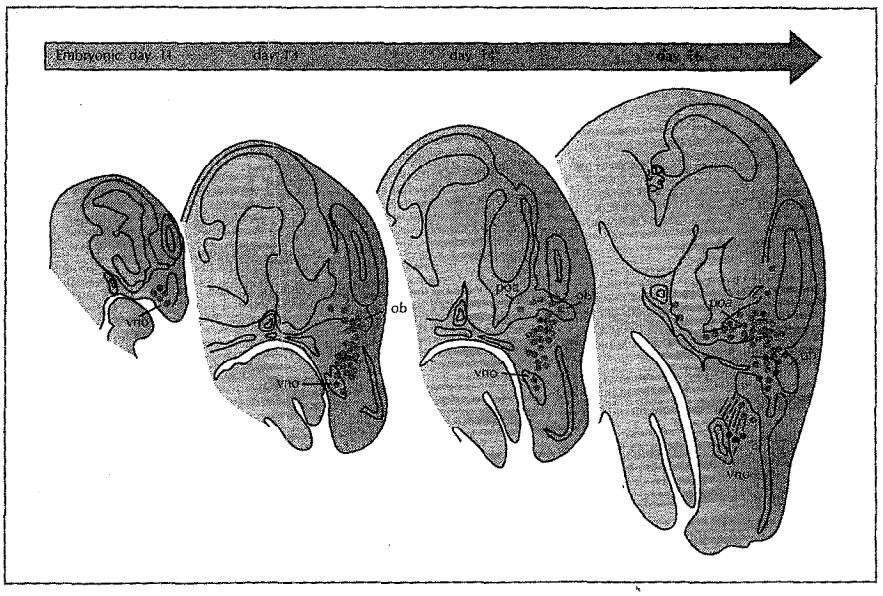

Fig. 1. Sagittal sections through the whole heads of developing mice, showing migration of LHRH-expressing neurons (colored purple) from the developing olfactory epithelium of the nasal cavity to the brain during embryonic development. (Adapted from [1].)

by another group used pulsed-field gel electrophoresis to analyse DNA from an additional patient with a terminal deletion of the short arm of the X-chromosome (patient LIL 155), and was able to map *KAL* to an interval of about 350 kilobases (kb) in this region [7] (Fig. 2).

Both groups have now isolated cDNA clones corresponding to a candidate for the Kallmann Syndrome gene product, called *KALIG-1* by one group [8] and *ADMLX* (for adhesion molecule-like from the X chromosome) by the other [9]. YAC (yeast artificial chromosome) clones from human genomic YAC libraries were used as a starting point for the derivation of genomic probes (subcloned from the YACs into λ phage vectors) that span the *KAL* interval. Franco *et al.* [8] used these probes to screen 'zoo blots' of DNA from distantly related mammalian species to identify genomic sequences that are evolutionarily conserved. Conserved sequences were then used to screen cDNA libraries made from human fetal tissue and a teratocarcinoma cell line, and overlapping clones were used to establish the sequence for the *KALIG-1* gene, which is predicted to encode a protein product of 680 amino acids. Southern blots of DNA from Kallmann Syndrome patients with Xp22.3 deletions and translocations, screened with both a genomic fragment containing the last 3′ exon of *KALIG-1* and cDNA probes spanning several exons of the gene, showed abnormal patterns of hybridization. This indicates that the chromosomal breakpoints in these patients reside in the *KALIG-1* gene, strong support for the hypothesis that *KALIG-1* is the gene deleted in X-linked Kallmann Syndrome. The authors added a note in proof, stating their discovery of an intragenic deletion in the *KALIG-1* gene of a patient with isolated Kallmann Syndrome (that is, hypogonadotropic hypogonadism and anosmia not associated with the other inherited syndromes that map to the distal short arm of the X chromosome), providing further evidence in support of the hypothesis that *KALIG-1* is the Kallmann Syndrome gene.

In their approach, Legouis and colleagues [9] first isolated and characterized a single YAC spanning the entire previously-defined *KAL* interval (YAC 376 B4), using a DNA probe (CRI-S232) that detects a locus that is inside the interval, but whose deletion is not associated with Kallmann Syndrome. They then redefined the *KAL* interval to the region limited proximally by the X/Y translocation breakpoint of patient AM and limited distally by the locus defined by CRI-S232 (Fig. 2). Fragments of the YAC spanning this *KAL* interval were subcloned into a λ phage vector, and overlapping phage clones covering the newly-defined (67 kb) interval were isolated and sequenced. Computer analysis of the sequence was used to define putative exons. Sequences derived from these exons were used to probe 'zoo blots', and one evolutionarily conserved sequence was used to probe cDNA libraries generated from human and monkey fetal brain. A cDNA sequence, containing a single long open reading frame of 679 amino acids, was obtained from 7 partially overlapping cDNA clones. The predicted protein is identical to the *KALIG-1* candidate gene product identified by Franco *et al.* [8].

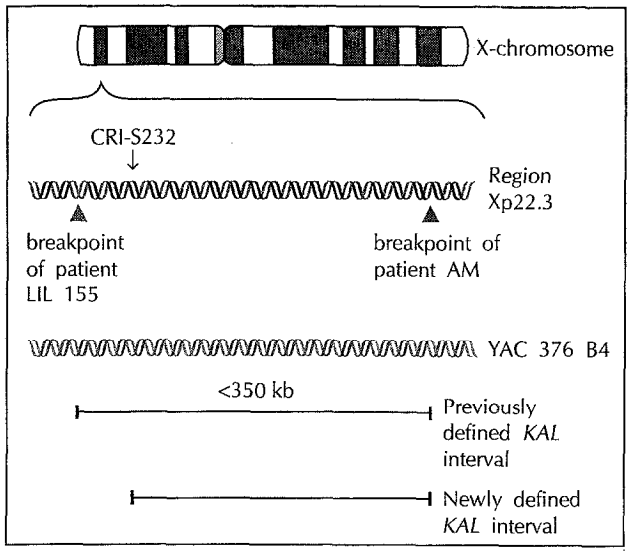

Fig. 2. Schematic representation of *KAL* intervals. The intervals defined by the breakpoints in patients LIL 155 and AM [7], and by probe CRI-S232 and breakpoint AM [9], respectively, are indicated at the bottom. Patient AM is affected by Kallmann Syndrome plus four other disorders mapping to Xp22.3; patient LIL155 is not affected by Kallmann Syndrome, but is affected by the other four disorders. (Adapted from [9].)

The cDNAs sequenced by both groups predict a protein whose absence presumably accounts for both the hypogonadotropic hypogonadism and anosmia that afflict males with X-linked Kallmann Syndrome. In sequence comparisons, the *KALIG 1/ADMLX* gene shows the greatest degree of sequence similarity to a class of genes encoding cell adhesion receptors in the immunoglobulin superfamily. Members of the immunoglobulin superfamily class of cell adhesion receptors are thought to be of great importance in neuronal migration and axon outgrowth during nervous system development. Most of these molecules are thought to mediate cell–cell (or axon–axon) adhesion [10].

It is not difficult to imagine that a cell–cell adhesion protein could be critical both for proper migration of LHRH neurons during development, and for the outgrowth of axons from olfactory receptor neurons thought to be crucial to the development and maintenance of the sense of smell. However, identification of the *KALIG-1/ADMLX* gene product as a cell–cell adhesion molecule is still quite tentative. Its similarity to such molecules lies in the homology between its fibronectin type III repeats, and those contained by a number of neuronal cell adhesion molecules of the immunoglobulin superfamily (TAG-1, L-1, F11/F3/contactin). But fibronectin type III repeats are also found in molecules of the extracellular matrix, including both fibronectin and tenascin (also known as hexabrachion and cytotactin). Both of these molecules are also known to regulate adhesive and migratory behaviors of neural cells [11]. As the *KALIG-1/ADMLX* sequence contains an N-terminal hydrophobic leader peptide, but no sequences consistent with its possessing a transmembrane domain or phosphatidyl inositol anchorage, it is probably a secreted protein. It could, therefore, function as a substrate adhesion molecule in the man-

ner of extracellular matrix glycoproteins. In this light, it is interesting that low-level expression of *KALIG-1/ADMLX* was detected in every tissue tested, including brain, muscle, kidney, liver, gut and lymphoid cells [3,9]. The extracellular matrix molecules known to function in neuronal migration and axon outgrowth are also widely expressed in both neural and non-neural tissues, whereas many neuronal cell adhesion molecules of the immunoglobulin superfamily show a much more restricted, nervous-system-specific pattern of expression [10,11].

Assessment of the function of the *KALIG-1/ADMLX* gene now awaits a number of crucial advances, the first of which must be identification of a transcript corresponding to the predicted gene product. Neither group has yet reported detection of a transcript on northern blots, presumably because of low levels of expression in the tested tissues. Expression of the *KALIG-1/ADMLX* gene product could only be detected by reverse transcription of RNAs from various tissues, followed by PCR (polymerase chain reaction) amplification [8,9]. Once the protein can be expressed and purified, however, it can be tested for a potential role in cell adhesion, migration, and/or neuronal axon outgrowth. An *in vitro* system for studying these processes in olfactory receptor neurons and neuronal precursors has recently been described [12] and may facilitate these studies.

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