

# Kallmann Syndrome: Adhesion, Afferents, and Anosmia

## Minireview

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Three new studies into the function of human anosmin-1 and related proteins in *C. elegans* and rodents show that these influence axon branching and axon targeting. The rodent anosmin appears to work at two stages of development, initially promoting axon outgrowth from the olfactory bulb and then stimulating branching from axons into the olfactory cortex. *CeKal-1* further influences morphogenesis, and, as the human and nematode anosmins are functionally conserved, these studies provide insights into the pathogenesis of Kallmann syndrome (KS).

### *The Biology of Kallmann Syndrome*

Kallmann syndrome (KS) is characterized by isolated gonadotrophin deficiency (IHH) and anosmia (a loss of sense of smell) and is a genetically heterogeneous condition, with X-linked, autosomal dominant and recessive modes of inheritance. X-linked Kallmann syndrome (X-KS) typically presents with IHH and anosmia, bimanual synkinesis (upper body mirror movements), and urogenital defects including renal agenesis.

Bimanual synkinesis is a defining feature of X-KS and appears to be caused by misrouting of descending corticospinal tract axons. Electrophysiological studies of X-KS patients with synkinesis, using crosscorrelation analysis of surface electromyograms and electromagnetic stimulation of the motor cortex hand areas, have revealed fast conducting bilateral projections from each motor cortex—inferring the presence of a novel ipsilateral corticospinal tract. Indeed, voxel-based morphometric analysis of pooled MR images has demonstrated bilateral hypertrophy of the corticospinal tracts in X-KS patients compared with autosomal KS and normal controls (Krams et al., 1997, 1999; Mayston et al., 1997).

The autosomal forms of KS (A-KS) exhibit no consistent phenotype apart from gonadotrophin deficiency and anosmia, although a heterogeneous collection of defects are seen in a minority of cases (for instance, midline developmental defects, including cleft lip and palate, other craniofacial anomalies, coloboma, and sen-

sorineural deafness) (Oliveira et al., 2001; Quinton et al., 2001). The genes causing A-KS have proven difficult to identify by conventional techniques, as patients tend to be infertile and pedigrees are thus invariably small. Hence, the main focus of research into the origins of KS has instead concentrated on the gene causing X-KS (*KAL1*) and the protein this encodes (anosmin-1), a modular extracellular matrix protein of about 100 kDa, consisting of an N-terminal cysteine-rich domain, followed by a WAP domain, four FnIII domains, and a C-terminal histidine-rich region (Figure 1). The combination of WAP and FnIII domains are unique to anosmin-1 and the 4-disulphide core WAP domain is similar to a number of protease inhibitors (<http://smart.embl-heidelberg.de>). The FnIII domains of anosmin-1 are related structurally to members of the neural cell adhesion molecule (N-CAM) protein family, including membrane-bound proteins such as TAG-1 and L1 (Robertson et al., 2001).

During human embryonic development, anosmin-1 and *KAL1* mRNA are found in various tissues affected in X-KS (Hardelin et al., 1999). These include the developing motor neurons of the spinal cord; the kidney mesonephros and metanephros; the presumptive olfactory bulb and later mitral cell layer; the contact areas where olfactory, vomeronasal, and terminal nerve axons and GnRH1 neurons penetrate the forebrain; and also the intracerebral migratory route of GnRH1 cell bodies.

### *Loss of GnRH1 and Olfactory Function in KS*

“Sex and smell” are linked in many species, and in primates the main population of GnRH1 neurons, which control activity of the reproductive axis, originate in the peripheral olfactory system. When the *KAL1* gene is disrupted, migratory GnRH1 cells become trapped in the nasal cavity, and no GnRH1 immunoreactivity is seen in the CNS. This is combined with a defective underlying cranial nerve 1 elongation pathway; where the axon terminals of the olfactory, terminalis, and vomeronasal nerves become trapped below the forebrain, with conspicuous absence of the olfactory bulbs (Schwanzel-Fukuda, 1999). Low or absent gonadotrophin (LH and FSH) and inhibin B levels also indicate a hypothalamic GnRH1 deficiency having occurred during development (Oliveira et al., 2001; Pitteloud et al., 2002), and nasal epithelial biopsies from KS patients reveal the presence of GnRH1-immunoreactive neurons within an immature olfactory neurepithelium—reflecting disconnection from the olfactory bulbs (Schwob et al., 1993; Quinton et al., 1997). Magnetic resonance imaging (MRI) further shows defects in the olfactory bulbs/sulci, with reduced volume of the olfactory cortex (Quinton et al., 1996; MacColl et al., 2002).

### *Anosmin-1 Promotes Axon Branching and Outgrowth*

How does loss of *KAL1* cause these defects in the olfactory system? The new study by Soussi-Yanicostas et al. (2002) demonstrates that anosmin-1 stimulates afferent projections from the mitral/tufted cell layer of the bulb, which develop into the lateral olfactory tract (LOT) connecting the olfactory bulb and cortex. Using monoclonal and polyclonal antibodies generated against human an-

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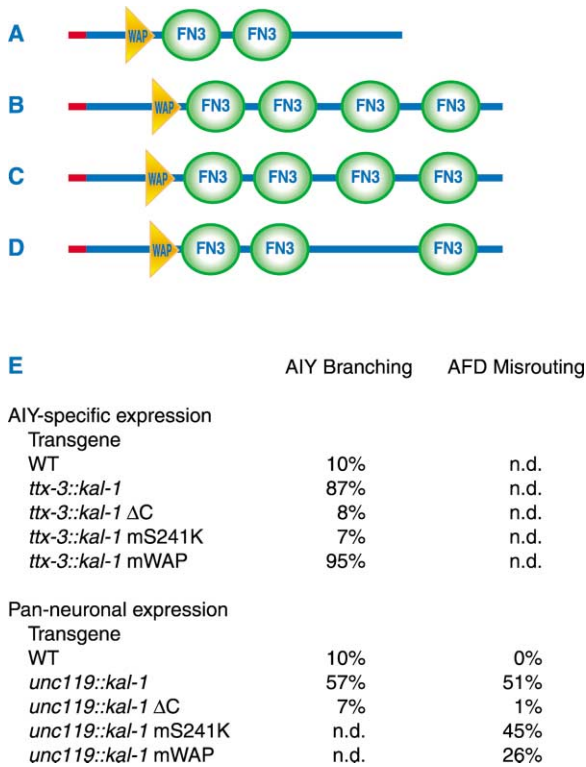


Figure 1. Structures of Vertebrate and Invertebrate “Anosmins” and Neuronal Overexpression Phenotypes in *C. elegans*

(A–D) SMART analysis of WAP and FN3 domain for vertebrate and invertebrate “anosmins” (<http://smart.embl-heidelberg.de>). (A) *D. melanogaster* KAL-1 (sptremblnew AAL73340) (B) *H. sapiens* KAL-1 (swissnew P23352) (C) *C. elegans* KAL-1 (sptremblnew AAL73338) (D) *G. gallus* KAL-1 (sptrembl QPSH7). Adapted, with permission, from several figures based on proteins from the SMART site, <http://smart.embl-heidelberg.de>.

(E) *CeKal-1* neuronal overexpression phenotypes using three mutants comparable to X-KS mutations: *kal-1* ΔC = deletion of C-terminal FnIII domains *kal-1* mS241K = FnIII-1 point mutant, *kal-1* mWAP = double WAP mutant (with C134S, C135S). Adapted from Bülow et al. (2002), National Academy of Sciences, USA.

osmin-1, an “anosmin” of about 100 kDa was detected in the developing olfactory system of both rats and mice (Soussi-Yanicostas et al., 1996, 2002). The rat anosmin is distributed throughout the mitral cell layer of the bulb, the lateral olfactory tract, and olfactory cortex and has two distinct effects on explants of these tissues cultured in vitro. First, olfactory bulb axons grow preferentially toward piriform cortex and also to CHO cells secreting human anosmin-1. Second, neurite branching is induced by anosmin-1 in dissociated olfactory bulb cultures. Antibodies generated against anosmin-1 inhibited both the chemoattractive and branching effects of anosmin-1 and inhibited collateral branching of E17 LOT axons, which would normally project into the olfactory cortex. Furthermore, olfactory bulb neurons become responsive to anosmin-induced branching activity after E15 in the rat, suggesting the receptor and/or signaling pathway through which the rodent anosmin interacts becomes active at this later stage of olfactory development (Soussi-Yanicostas et al., 2002). Anosmin-1 therefore

influences the development of both the primary and secondary olfactory processing regions — both of which are impaired in X-KS.

#### Cross-Species Anosmins

A more detailed genetic analysis of rodent anosmin function and its interacting factors is not possible at present, as the mouse and rat *Kal1* genes have not yet been described (Soussi-Yanicostas et al., 2002). However, cross-species analysis of *Kal1* genes has revealed sequences in a variety of vertebrates and invertebrates, ranging from model organisms such as *Caenorhabditis* and *Drosophila*, through to fugu, quail and chick, and zebrafish. The activity of these “anosmins” also resides in a multidomain protein consisting of a WAP domain, followed by a variable number of FnIII domains (Figure 1). The first systematic analyses of anosmin function has been carried out in *C. elegans*, and remarkably the nematode *Kal1* ortholog (*CeKal-1*) also induces dosage-dependent changes in neurite branching and in axon outgrowth, both in loss-of-function mutants and when *CeKal-1* is overexpressed in a number of neuronal subtypes (Rugarli et al., 2002; Bülow et al., 2002) (Figure 1).

What components of anosmin-1 determine these activities? Using three mutants that recapitulated X-KS mutations (Oliveira et al., 2001), Bülow et al. have shown that the *CeKal-1* WAP and FnIII domains appear to have different functions with respect to the neuronal overexpression phenotypes (Figure 1). Expression of a truncated *CeKal-1* missing most of the FnIII domains (*kal-1* ΔC) did not induce branching in neurons and abolished the axon misrouting phenotype. A second *CeKal-1* mutation in the 1<sup>st</sup> FnIII domain (*kal-1* mS241K) also abolished branching, but had no effect on axon targeting. The third mutant (*kal-1* mWAP) in the 4-disulphide core WAP domain diminished the mistargeting phenotype, but had no effect on axon branching (Bülow et al., 2002). Taken together, these results suggest that the WAP domain influences axon targeting only, and the FnIII domains are essential for this function and also for axon branching.

#### *CeKal-1* Controls Epithelial Morphogenesis in *C. elegans*

*CeKal-1* is also required for ventral enclosure during embryogenesis and is first detected in a group of neuroblast descendants of the AB blastomere, which act as a substrate for epidermal migration following gastrulation (Rugarli et al., 2002). Visualization of epidermal cell boundaries in comma-stage embryos demonstrates abnormal positioning between cells, both in loss-of-function mutants and in worms overexpressing *CeKal-1*—suggesting these cells migrate to surround the embryo during morphogenesis but fail to form reciprocal adherent contacts. Loss of *CeKal-1* function generates a number of striking phenotypes in mutants including ventral enclosure and ray (tail) abnormalities in males (Rugarli et al., 2002), and, although it is not clear whether *CeKal-1*-expressing neuroblasts provide an active signal or permissive substrate for migrating epithelial cells (Chin-Sang and Chisholm, 2000), vertebrate anosmin-1 can form adhesive contacts with mammalian epithelial cells (Soussi-Yanicostas et al., 1998; Robertson et al., 2001). Significantly, *CeKal-1* mutants could also be rescued with the human or *C. elegans* *KAL1* sequences indicat-

ing the function of both anosmins are conserved (Rugarli et al., 2002).

#### **What Factors Influence *CeKal-1* Function in *C. elegans*?**

In *C. elegans*, ephrin/EphR and semaphorin signaling pathways are central to neurite branching and for axon guidance, in addition to the cell movements and interactions that occur during morphogenesis (Chin-Sang and Chisholm, 2000). Yet the most penetrant axon branching phenotype observed in worms overexpressing *CeKal-1* in AIY neurons was largely unaffected in mutant backgrounds for Eph-R and Semaphorin, in addition to other genes including Netrin, Robo, FGFR, integrin, and several other ECM molecules (Bülow et al., 2002). Expression in mutant backgrounds for two  $\beta$ -spectrins also showed a higher suppression of phenotype (29%–36%); *unc-70* may be involved in the localization of cell adhesion/signaling molecules (Hammarlund et al., 2000) and requires further investigation. However, a modifier screen to isolate new mutations in genes required for *CeKal-1* to exert its function demonstrated almost complete suppression of *CeKal-1*-induced branching of AIY neurons in a heparan-6-O-sulfotransferase-deficient background (Bülow et al., 2002)—thus confirming that interaction of anosmin with HSPG at the cell surface/ECM is essential for its biological activity (Soussi-Yanicostas et al., 1996). A number of other modifier loci were also identified and are currently being investigated (Bülow et al., 2002).

#### **Conclusions**

These studies on anosmin function in rodents and in *C. elegans* provide a wealth of new information, both on the development of the CNS structures that process olfactory information, as well as on the development of neuronal structures and cell–cell interactions required for morphogenesis in *C. elegans*. A key observation is the conservation of function between *CeKal-1* and human *KAL1*—both rescue lethality in *CeKal-1* mutants. Both also influence axon targeting and branching, and Bülow et al. took this one step further—showing the WAP and FnIII domains of *CeKal-1* had different properties with respect to each of these phenotypes (Bülow et al., 2002). This study also showed the axon branching and misrouting function to be dependent on interactions with heparan sulfate.

Based on the above studies, how could a mutation in the human *KAL1* gene account for the full phenotype of X-KS—IHH and anosmia, upper body mirror movements, and renal agenesis? Soussi-Yanicostas et al. show that anosmin-1 affects axon outgrowth from the olfactory bulb and later branching, when these project to the olfactory cortex. A loss of anosmin-1 function in these regions would be consistent with defects observed in both primary and secondary olfactory processing areas in X-KS patients with anosmia. However, this only tells half the story as anosmia is coupled to gonadotrophin deficiency in X-KS. It also appears that *KAL1* is necessary for contacts to occur between olfactory and vomeronasal axons and the olfactory bulb and between adjacent regions of the telencephalon (Schwanzel-Fukuda, 1999; Hardelin et al., 1999). These axons form the tracks along which GnRH1 neurons migrate to the brain, and, in addition to being necessary for olfactory bulb development, loss of this pathway

could lead to a deficiency of hypothalamic GnRH1 neurons and subsequently cause IHH.

With respect to the other X-KS phenotypes, axon misrouting in the absence of anosmin-1 could also cause the apparently irregular development of the corticospinal tracts seen in X-KS (Krams et al., 1997, 1999; Mays-ton et al., 1997). In addition, the asymmetrical contacts between cells in *CeKal-1* mutants may provide a link to the abnormal kidney morphogenesis seen in significant number X-KS pedigrees. A number of other questions also remain unanswered. (1) What are the elusive factors that interact with anosmin-1, in addition to heparan sulfate/HSPG? (2) Are separate signaling pathways required for neuronal development and for the morphogenetic actions of the *C. elegans* anosmin? (3) What are the identities of the additional genes that cause autosomal-KS? Notably, A-KS is also not an “all or none” condition, as IHH and anosmia can occur together and separately within the same pedigree—thus development of the olfactory bulb may not always be essential for GnRH1 development in humans (Quinton, et al., 2001). Further studies of *KAL1* function in rodents and in model organisms such as *C. elegans* will enable the identification of the genes responsible for A-KS.

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