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A six-gene expression toolbox for the glands, epithelium and chondrocytes in the mouse nasal cavity

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ABSTRACT

The nose is the central feature of the amniote face. In adults, the nose is a structurally and functionally complex organ that consists of bone, cartilage, glands and ducts. In an ongoing expression screen in our lab, we found several novel markers for specific tissues in the nasal region. Here, using *in situ* hybridization expression experiments, we report that *Alx1*, *Ap-2β, Crispld1, Eya4, Moxd1,* and *Penk* have tissue specific expression during murine nasal development. At E11.5, we observed that *Alx1*, *Ap-2β*, *Crispld1*, and *Eya4* are expressed in the medial and lateral nasal prominences. We found that *Moxd1* and *Penk* are expressed in the lateral nasal prominences. At E15.5, *Alx1* is expressed in nasal septum. *Ap-2β* and *Crispld1* are expressed in nasal glands and cartilages. *Eya4* is expressed in olfactory epithelium. Intriguingly at E15.5 *Moxd1* is expressed in all the nasal cartilage while the expression of *Penk* is restricted to chondrocytes contributing to the posterior nasal septum. The expression domains reported here suggest that these genes warrant functional studies to determine their role in nasal capsule morphogenesis.

1. Introduction

In adults the nasal region can be divided into three distinct zones: the vestibule, respiratory region and olfactory region. The nasal vestibule is the most rostral region and consists of the nasal aperture (nostrils) and is lined with a stratified squamous epithelium (Chamanza and Wright, 2015; May and Tucker, 2015). The respiratory region is lined with respiratory epithelium (RE) and functions to moisten and protect the nasal cavity. The start of the olfactory region is demarcated by the sharp transition from RE to olfactory epithelium (OE). The olfactory region is lined with OE is composed of olfactory sensory neurons, supporting cells, and basal cells and functions to distinguish and detect odors (Huard et al., 1998; Kawauchi et al., 2005; Maurya et al., 2015; Ressler et al., 1993; Vassar et al., 1993). The nasal cavity also contains several types of secretory glands, including lateral and medial glands, which secrete mucus into the nasal cavity where it traps airborne particles within inspired air, preventing them from progressing to the lower airway tubes and lungs (May and Tucker, 2015; May et al., 2016).

The hard tissue of the nasal capsule consists of paired nasal cavities are surrounded by bone and cartilage and lined with respiratory and olfactory epithelia. The midline of the nasal capsule consists of a cartilaginous nasal septum that is surrounded by nasal turbinates (conchae) that project from and are named for the bones that they originate from. In the mouse, the nasal cavity is surrounded anteriorly by the nasal bones and laterally by the maxillary bones, and caudally by the ethmoid and ventrally by the vomer.

In an ongoing expression screen of genes expressed in the craniofacial region, we observed expression of *Alx1*, *Ap-2β*, *Crispld1, Eya4, Moxd1* and *Penk* in the embryonic nasal capsule. *Alx1* (ALX homeobox 1) is a paired-type homeobox-containing transcription factor involved in craniofacial and neural tube development in cat, mice and humans (Bhattacherjee et al., 2009; Uz et al., 2010; Zhao et al., 1996). The *Alx1*$^{-/-}$ knockout mouse has a malformed nasal septum at birth (Qu et al., 1999; Zhao et al., 1996). A 12- basepair in-frame deletion in *ALX1* is concordant with a severe frontonasal dysplasia phenotype, in Burmese Cats (Lyons et al., 2016). In Darwin's finches, the *Alx1* locus is

Abbreviations: d, ducts; e, eye; lng, lateral nasal glands; lnp, lateral nasal prominence; lt, lateral turbinate; mdp, mandibular prominence; mnp, medial nasal prominence; mxp, maxillary prominence; n, nasal bone; ns, nasal septum; oe, olfactory epithelium; om, ocular muscles; pc, perichondrium; re, respiratory epithelium; v, vestibule; vi, vibrissae; vn, vomeronasal organ; zof, zone of fusion

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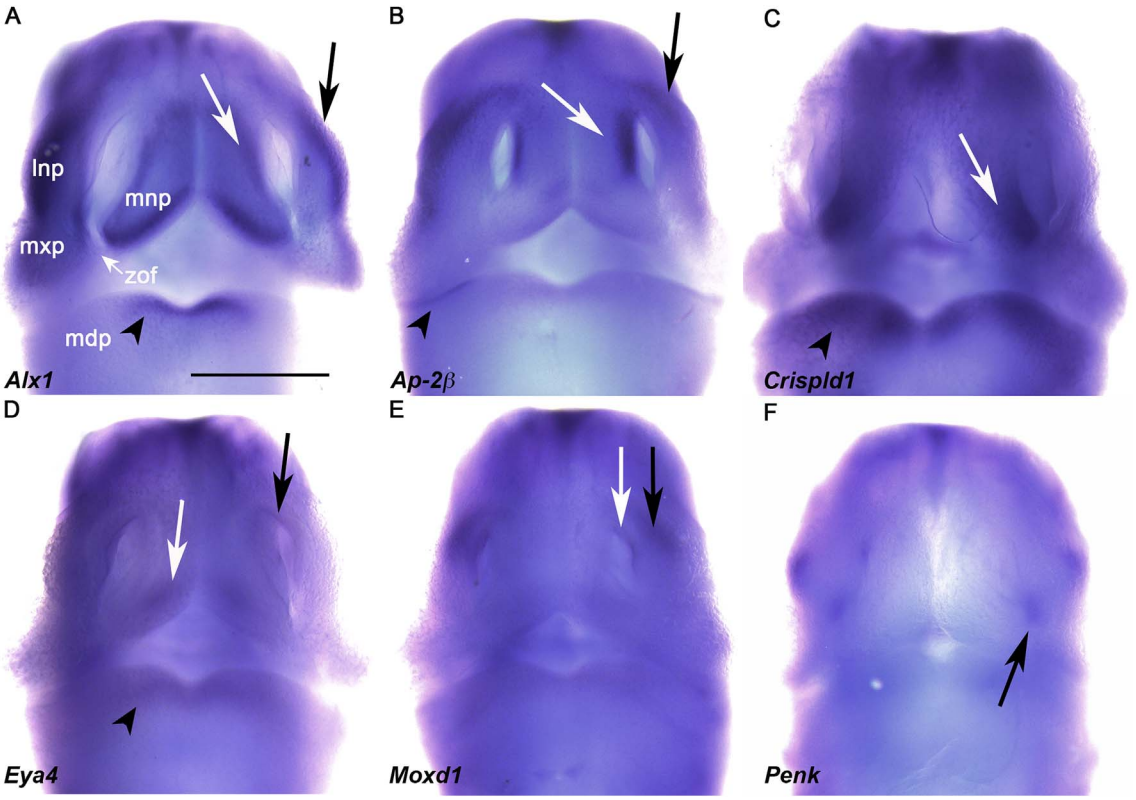

Fig. 1. *Alx1*, *Ap-2β*, *Crispld1*, *Eya4*, *Moxd1* and *Penk* are expressed in the nasal region at E11.5. A) *Alx1* is expressed in the periphery of the lnp (black arrow), mnp (white arrow), and medial mdp (arrowhead). B) *Ap-2β* is expressed in the lateral edge of the rostral mnp (white arrow), overlapping *Alx1* expression in the mnp. *Ap-2β* is expressed in the lateral mdp (arrowhead), and the lnp (black arrow). C) *Crispld1* is expressed in the caudal region of the mnp (white arrow), where *Alx1,* and *Ap-2β* is not expressed. D) *Eya4* is expressed in the nasal pit epithelium (black arrow) medial mnp (white arrow) and the entire medial-lateral axis of the mdp (arrowhead). E) *Moxd1* is expressed in the nasal pit epithelium (white arrow), and the lateral lnp (black arrow). F) *Penk* is expressed near the zof (black arrow). Scale bar = 100 μm and applies to all.

associated with variations in beak morphology (Lamichhaney et al., 2015). *Ap-2β* (Transcription factor *Ap-2β*) belongs to a family of five transcription factors, *Ap-2α*, *Ap-2β* , *Ap–2δ*, Ap–2γ, Ap–2ε that are expressed in epithelial and neural crest cell lineages during craniofacial and skeletal development. *AP-2β*$^{-/-}$ knockouts defects in the neural tube, renal, lung and bone (Moser et al., 2003; Schorle et al., 1996; Seki et al., 2015; Wenke and Bosserhoff, 2010; Zhang et al., 1996; Zhao et al., 2011). *Crispld1* (cysteine-rich secretory protein LCCL domain containing 1) is an extracellular matrix protein. In tissue culture models of osteoarthritis, *Crispld1* is enriched in the IL-1α treated samples (Wilson et al., 2016). Single nucleotide polymorphisms in human *CRISPLD2* are linked to human orofacial clefting (Chiquet et al., 2007, 2011; Gibbs et al., 2008). *Eya4 (*Eyes absent homolog 4) is one member of the *Eya* gene family. Homozygous null mice have inherited otitis media, defects in palatal and skull bone morphology and defects in eye development (Depreux et al., 2008; Pignoni et al., 1997). *Moxd1* (Monooxygenase, DBH-like 1) belongs to the copper-dependent monooxygenase protein family. *Moxd1* is a marker for sexual dimorphic nuclei in the pre-optic area, the bed nucleus of the stria terminalis and amygdala (Tsuneoka et al., 2017). Proenkephalin (*Penk*) is peptide that has opioid activity and is expressed by the adrenal glands, the knockout mice are viable and fertile, with no reported craniofacial phenotype (Konig et al., 1996).

Here we report the complimentary and overlapping tissue specific expression domains of *Alx1*, *Ap-2β*, *Crispld1, Eya4, Moxd1* and *Penk* in the mouse nasal capsule.

2. Results

2.1. Expression of Alx1, Ap-2β, Crispld1, Eya4, Moxd1 and penk in E11.5 faces

The development of the nasal region is well established by E11.5, the nasal pit has begun to invaginate into the craniofacial mesenchyme, two species of epithelium, the respiratory and olfactory epithelium is present, and the primary palate has fused between the medial nasal, lateral nasal and maxillary prominences. We first wanted to establish whether our genes of interest are expressed at this stage. We observed these six genes expressed at E11.5 by whole-mount *in situ* hybridization. *Alx1* is expressed in caudal to rostral regions of the medial nasal prominence (mnp) (Fig. 1A, white arrow), and lateral nasal prominence (lnp) (Fig. 1A, black arrow), but is excluded rostrally above the nasal pits. *Ap-2β* expression domain is connected rostrally above the nasal pits and connects the lnp expression zone with the mnp expression zone (Fig. 1B, arrows). *Ap-2β* is also expressed strongly on the lateral edges of the mnp and medial regions of the lnp (Fig. 1B, white arrow).