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# Expression of LIM-homeodomain transcription factors in the developing and mature mouse retina

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ABSTRACT

LIM-homeodomain (LIM-HD) transcription factors have been extensively studied for their role in the development of the central nervous system. Their function is key to several developmental events like cell proliferation, differentiation and subtype specification. However, their roles in retinal neurogenesis remain largely unknown. Here we report a detailed expression study of LIM-HD transcription factors LHX9 and LHX2, LHX3 and LHX4, and LHX6 in the developing and mature mouse retina using immunohistochemistry and *in situ* hybridization techniques. We show that LHX9 is expressed during the early stages of development in the retinal ganglion cell layer and the inner nuclear layer. We also show that LHX9 is expressed in a subset of amacrine cells in the adult retina. LHX2 is known to be expressed in retinal progenitor cells during development and in Müller glial cells and a subset of amacrine cells in the adult retina. We found that the LHX2 subset of amacrine cells is not cholinergic and that a very few of LHX2 amacrine cells express calretinin. LHX3 and LHX4 are expressed in a subset of bipolar cells in the adult retina. LHX6 is expressed in cells in the ganglion cell layer and the neuroblast layer starting at embryonic stage 13.5 (E13.5) and continues to be expressed in cells in the ganglion cell layer and inner nuclear layer, postnatally, suggesting its likely expression in amacrine cells or a subset thereof. Taken together, our comprehensive assay of expression patterns of LIM-HD transcription factors during mouse retinal development will help further studies elucidating their biological functions in the differentiation of retinal cell subtypes.



The mammalian neural retina is comprised of six major neuronal cell types and one glial cell type. Structurally, the retina can be divided into three layers: the outer nuclear layer (ONL) containing cell bodies of rod and cone photoreceptors, the inner nuclear layer (INL) containing cell bodies of horizontal cells, bipolar cells, amacrine cells and Müller glial cells, and the ganglion cell layer (GCL) containing cell bodies of retinal ganglion cells and displaced amacrine cells (Livesey and Cepko, 2001; Masland, 2001a; Hatakeyama and Kageyama, 2004). Retinal neurons further display heterogeneity in morphology and functions in visual signal processing and are hence further classified into retinal cell subtypes (Masland, 2001b). For instance, bipolar cells are classified into rod and cone bipolar cells depending on the photoreceptor type they receive their synaptic input from, and further as ON and OFF bipolar cells based on their polarizing response to light stimulus (Masland, 2012a). Amacrine cells have also been classified into different categories based on the neurotransmitter type they express (Masland, 2012b). Amacrine cells can be GABAergic or Glycenergic or as recently discovered, neither (nGnG) (Kay et al., 2011). They can further be classified as cholinergic, tyrosine hydroxylas-expressing or parvalbumin (PV)-expressing amacrine cells, to name a few (Wassle, 2004; Bhati et al., 2008; Voinescu et al., 2009). Several transmitter markers such as ChAT and GABA mark amacrine cell subtypes and markers such as PKCα and Goα distinguish between classes of bipolar cells (Wassle, 2004), but they do not facilitate the labeling of cells undergoing differentiation from a pool of progenitors. This emphasizes the need for developmental biomarkers that can specifically label different subtypes of retinal cells. Several developmental markers that label subtypes of amacrine cells such as *Isl1*, *Lmo4*, *Bhlhb5* (Feng et al., 2006; Elshatory et al., 2007a,b; Duquette et al., 2010) and subtypes of bipolar cells such as *Bhlhb4* and *Bhlhb5* (Bramblett et al., 2004; Feng et al., 2006) have been recently identified. While the list of retinal cell subtypes is still growing, molecular markers that can identify and track them developmentally are mostly undiscovered.

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LIM-homeodomain (HD) transcription factors are characterized by the presence of two protein binding zinc finger motifs, the LIM domains, located at the N-terminal of a central HD that specifically bind TAAT-containing DNA sequences. Owing to the presence of LIM domains, LIM-HD transcription factors also have the unique ability to form homomeric or heteromeric combinatorial complexes with other transcription factors (Bach, 2000; Bhati et al., 2008; Dawid et al., 1998). Several proteins belonging to the LIM-HD family of transcription factors have been studied for their roles during the specification and differentiation of several central nervous system neurons (Reviewed in Hobert and Westphal (2000) and Shirasaki and Pfaff (2002)).

The expression of some LIM-HD factors during retinogenesis has been previously studied. LHX1 (also known as LIM1), is expressed in horizontal cells and directs the migration of developing horizontal cells to their correct laminar position in the inner nuclear layer (Liu et al., 2000; Poche et al., 2007). The expression and function of the ISLET group of LIM-HD factors during retinogenesis has also been extensively studied. While ISL1 is expressed during retinal development and in retinal ganglion cells, cholinergic amacrine cells and ON-bipolar cells in the adult retina (Pan et al., 2008; Elshatory et al., 2007a,b), ISL2 is expressed only in post-mitotic retinal ganglion cells (Pak et al., 2004). However, the expression of other LIM-HD factors during retinal development, have not been thoroughly characterized. Here we report the expression patterns of five of the LIM-HD proteins during the development of retina in mice: LHX9 and LHX2 belonging to the APTEROUS group, LHX3 and LHX4 belonging to the LIM-3 group and LHX6 belonging to the LHX6/7 group.

LHX9 and LHX2 belong to the APTEROUS group of LIM-HD transcription factors. LHX9 expression has been described in the mammalian system central nervous system structures such as spinal cord neurons, diencephalon, telencephalic vessels and dorsal mesencephalon (Retaux et al., 1999; Bertuzzi et al., 1999). Recently, LHX9 was also found to be expressed in a subset of hypocretin neurons in the hypothalamus and to have a crucial role in regulating somnolence (Dalal et al., 2013). Further, combinatorial expression of LIM-HD factors LHX9 and LHX2 has been demonstrated during the development of several systems (Nakagawa and O'Leary, 2001; Wilson et al., 2008; Abellan et al., 2009; Peukert et al., 2011; Chatterjee et al., 2012).

The expression of LHX2 was first described during mammalian central nervous system development by Bourgouin et al. (1992) and Xu et al. (1993). Subsequently Porter et al. (1997) described the importance of LHX2 during the early development of the forebrain, eye and in erythrocyte development. LHX2 mutants are anophthalmic, display severe malformations of the cerebral cortex and are not viable since they present with defective erythropoiesis leading to severe anemia. Specifically, during eye development, LHX2 expression is noted in the optic vesicle as early as E8.5 and continues to be expressed in neural retinal progenitor cells (Porter et al., 1997). In the absence of LHX2, the specification of the optic vesicle occurs but development is arrested prior to the formation of optic cup (Porter et al., 2007, Yun et al., 2009; Hagglund et al., 2011). It has also been shown to interact with and activate other eye-field specific transcription factors. LHX2 interacts with PAX6, which in turn activates the expression of SIX6 in retinal progenitor cells (Tetreault et al., 2009). LHX2 has also been shown to link several extrinsic and intrinsic factors to co-ordinate multiple patterning events for the formation of the optic cup (Yun et al., 2009). Conditional disruption of *Lhx2* shows that the expression of *Lhx2* is not only necessary for optic cup formation but is also necessary for differentiation of the neuroretina (Roy et al., 2013) by facilitating a transition in competence states (Gordon et al., 2013).

LHX3 and LHX4 belong to the LIM-3 (*Drosophila*) group of LIM-HD proteins (Yamashita et al., 1997; Sharma et al., 1998; Hobert and Westphal, 2000). Pioneering work describing the combinatorial regulation of development by LHX3 and LHX4 has been widely studied in two systems: pituitary organogenesis (Sheng et al., 1997) and spinal cord motor neuron subtype development (Tsuchida et al., 1994; Sharma et al., 1998; Thor et al., 1999).

LHX6 expression was first reported in the first branchial arch and the developing medial ganglionic eminence of the basal forebrain by Grigoriou et al. (1998), suggesting that LHX6 along with its closely related LIM-HD member – LHX7 might have functional roles in development of craniofacial structures and the forebrain. Soon after, (Marin et al., 2000) provided further evidence implicating LHX6 along with LHX7 in the development of striatal

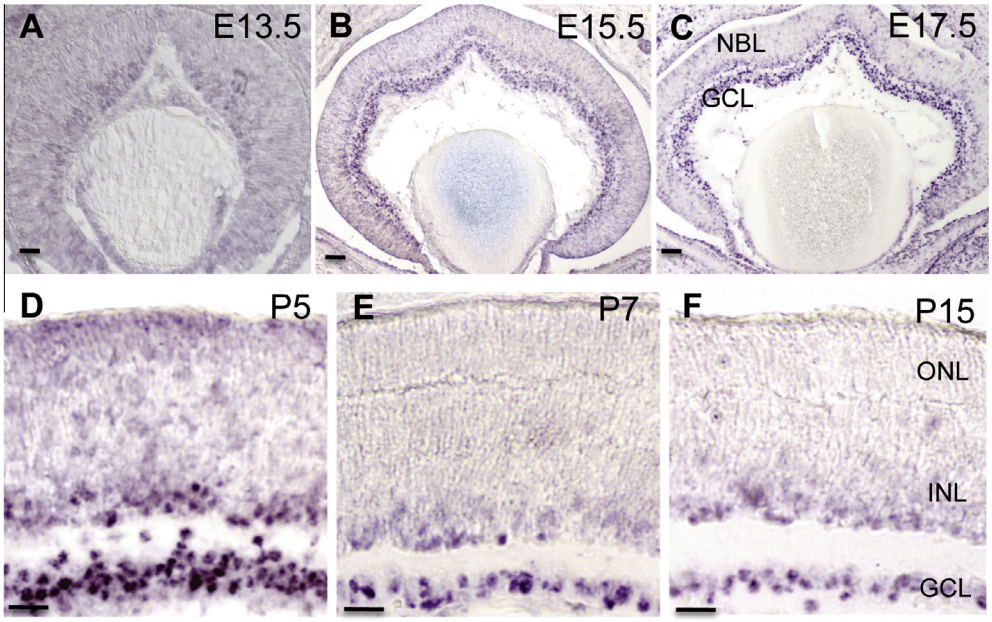


**Fig. 1.** Expression of *Lhx9* mRNA transcript in the developing retina. (A) Expression of *Lhx9* is faint and mostly undetectable at E13.5 in retinal progenitor cells. (B) Strong expression of *Lhx9* beings at E15.5 in the GCL and developing NBL. (C and D) Expression of *Lhx9* continues (E17.5, P5) in the GCL and developing NBL. (E and F): Expression of *Lhx9* is in cells in the GCL and cells in the INL postnatally (P7, P15). All scale bars are 50 μm.