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The expression of *Visinin-like 1* during mouse embryonic development

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ABSTRACT

Visinin like 1 (*Vsnl1*) encodes a calcium binding protein which is well conserved between species. It was originally found in the brain and its biological functions in central nervous system have been addressed in several studies. Low expression levels have also been found in some peripheral organs, but very little information is available regarding its physiological roles in non-neuronal tissues. Except for the kidney, the expression pattern of *Vsnl1* mRNA and protein has not yet been addressed during embryogenesis. By *in situ* hybridization and immunolabeling we have extensively analyzed the expression pattern of Vsnl1 during murine development. *Vsnl1* specifies the cardiac primordia and its expression becomes restricted to the atrial myocardium after heart looping. However, in the adult heart, Vsnl1 is expressed by all four cardiac chambers. It also serves as a specific marker for the cardiomyocyte-derived structures in the systemic and pulmonary circulation. Vsnl1 is dynamically expressed also by many other organs during development e.g. taste buds, cochlea, thyroid, tooth, salivary and adrenal gland. The stage specific expression pattern of Vsnl1 makes it a potentially useful marker particularly in studies of cardiac and vascular morphogenesis.

Visinin like 1 protein (VSNL1), also known as VILIP-1 or NVP-1, belongs to the Visinin-like protein subfamily of neuronal sensor calcium (NCS) proteins (Braunewell et al., 2001). They contain four EF-hand Ca^{2+} binding motifs, and most of them also a consensus sequence for N-terminal myristoylation. The Visinin subfamily comprises five members (VSNL1, VSNL2, VSNL3, Hippocalcin and Neurocalcin δ) with high homology in their amino acids sequences, 67–94% (Braunewell and Klein-Szanto, 2009). *Vsnl1* has been identified from several species including frogs (Klein et al., 2002), chicken (Lenz et al., 1992), fish and reptiles (Ren et al., 2010), rat (Kuno et al., 1992), mouse and humans (Polymeropoulos et al., 1995; Strausberg et al., 2002). The amino acid sequences are highly conserved between species (from 92.1% up to 100%) suggesting that selective pressure must have been extensively high for the conservation of VSNL1.

The functional data of VSNL1 are derived from *in vitro* studies. It is involved in regulation of cellular signaling cascades including cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP) signaling (Braunewell and Klein-Szanto, 2009). In pancreatic cells, VSNL1 regulates glucose-stimulated insulin secretion (Dai et al., 2006). In the central nervous system, VSNL1 specifically binds the 3′UTR of the neurotrophin receptor *trkB* RNA in a Ca^{2+}-dependent manner (Mathisen et al., 1999).

VSNL1 interacts with α4β2 nicotinic acetylcholine receptors (nAChR), and modulates their function and trafficking (Lin et al., 2002; Zhao et al., 2009). It also forms a signaling complex with P2X2 receptors and proteins such as *N*-ethylmaleimide-sensitive factor (NSF), tubulin1α, vesicle amine transport protein 1 homolog (VAT1), glutamic acid decarboxylase (GAD) and synapsin IIB (Chaumont et al., 2008).

The expression pattern of *Vsnl1* is well characterized in the adult brain (Paterlini et al., 2000; Zhao and Braunewell, 2008). In the rat, *Vsnl1* is abundant in all hippocampus regions and dentate gyrus. In humans, a high expression of *Vsnl1* is seen mostly in the hippocampus regions CA1, CA4 and hilus (Bernstein et al., 1999). In gerbils, VSNL1 immunoreactivity is highest in CA3 region (Lenz et al., 1996). In the chicken and rat cerebellum *Vsnl1* transcripts are restricted to the granular cells, and absent from the Purkinje cells (Lenz et al., 1992). In *Gekko japonicus*, *Vsnl1* is expressed in the gray matter and ependymal cells (Ren et al., 2010). In the retina of various species, VSNL1 is expressed in subsets of bipolar, amacrine and retinal ganglion cells (De Raad et al., 1995). Also, in rat olfactory epithelium a subset of neurons expresses VSNL1 (Bastianelli et al., 1995; Boekhoff et al., 1997).

Unlike the well described patterns in the brain, VSNL1 expression in non-neuronal organs is poorly characterized. Bioinformatics and

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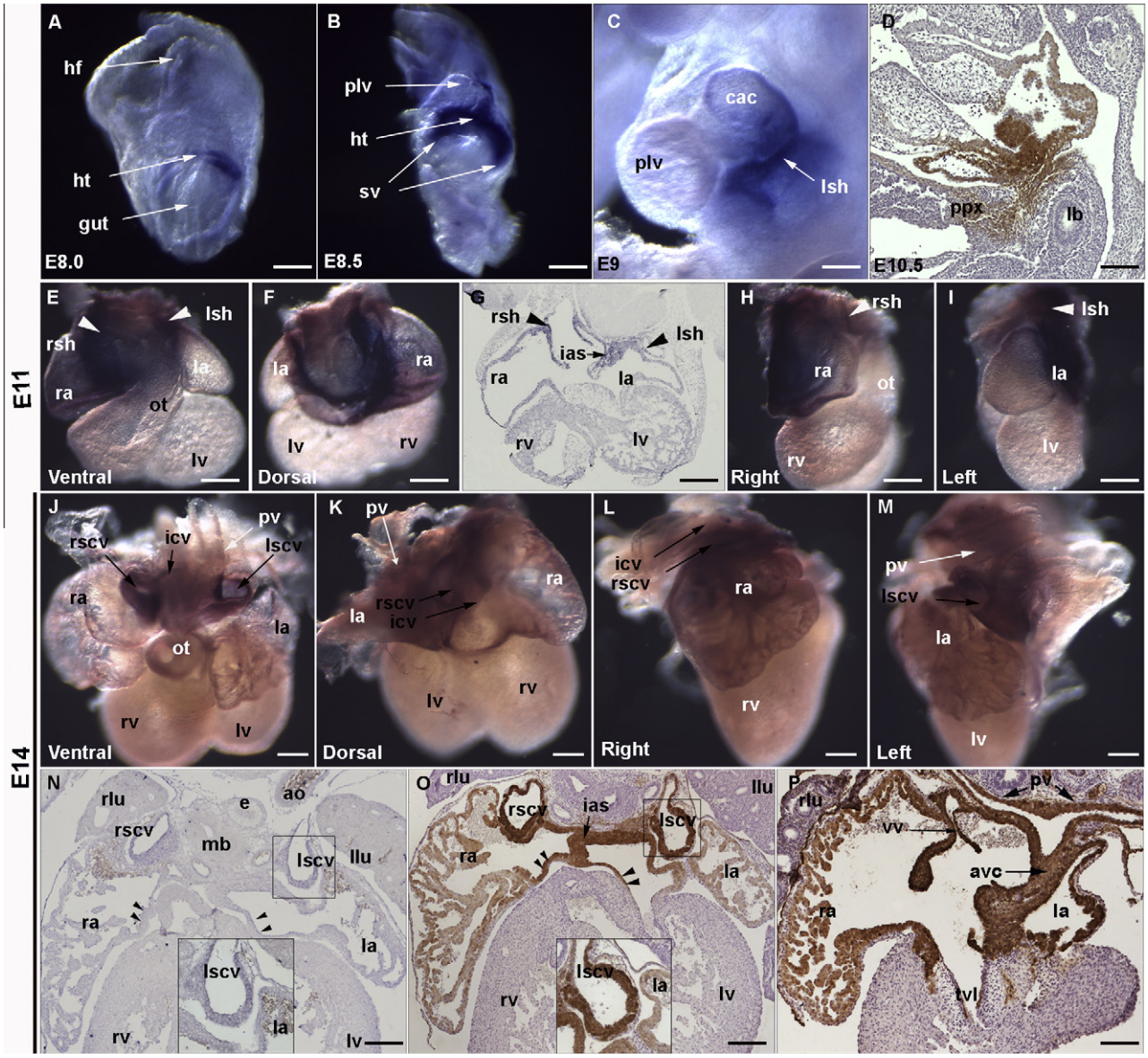

Fig. 1. *Vsnl1* mRNA and protein expression in early mouse heart development. *In situ* hybridization with a mRNA probe for *Vsnl1* in all figures excepting D,O,P. Ventral view of E8 (A), E8.5 (B) embryos and (C) left view of an E9 heart. (D) immunostaining of an E10.5 embryo sagittal section to visualize the pulmonary plexus expressing VSNL1. Whole amounts *in situ* hybritization on hearts dissected from E11 (E–I) and E14 (J–M) embryos viewed from ventral (E and J), dorsal (F and K), right (H and L) and left side (I and M). Tissue section of E11 (G) and E14 mouse hearts (N, O and P). *Vsnl1* transcript and the protein expression are confined to the atrial septum (arrows in G and O), sinus venosus (arrowheads in E, G, H and I), atria, caval veins (arrow in J–M), lower rim of the atrial appendages (arrowheads in N and O), venous valve leaflets (arrow in P), atrio-ventricular canal and pulmonary veins (arrows in P). Insets in N and O shows a detailed view of the cava vein and atrium. Scale bars: 100 μm. ao: aorta, avc: atrioventricular canal, cac: common atrial chamber, e: esophagus, hf: head folds, ht: heart tube, ias: atrial septum la: left atrium, lsh: left sinus horn, lb: lung bud, llu: left lung, lscv: left superior cava vein, lv: left ventricle, mb: main bronchus, ot: outflow tract, plv: primitive left ventricle, ppx: pulmonary plexus, pv: pulmonary vein, ra: right atrium, rlu: right lung, rscv: right superior cava vein, rsh: right sinus horn, rv: right ventricle, sv: sinus venosus, tvl: tricuspid venous valve, vv: venous valve leaflets.

western Blot analyses of embryonic and adult rat and human tissues, revealed different levels of Vsnl1 in heart, liver, lung, testis, kidney, spleen, ovary, pancreas, stomach, colon and skin (Ola et al., 2011; Gierke et al., 2004). Vsnl1 expression seems to be subject to developmental regulation in some of these organs and declines specifically in the adulthood which may hint to a specific role during organogenesis (Ola et al., 2011; Gierke et al., 2004). By immunohistochemistry (IHC), VSNL1 has also been detected in adult human esophagus (Wickborn et al., 2006) and pancreas (Dai et al., 2006). Furthermore, *Vsnl1* was identified by RT-PCR in adult murine gastrointestinal smooth muscle cells (Ohya and Horowitz, 2002). By western blot and RT-PCR analyses Vsnl1 has been found in the adult rat and human hearts where it regulates the natriuretic peptide receptor B (Buttgereit et al., 2010). To date, there are no transgenic models available for *Vsnl1* and consequently, the *in vivo* function of *Vsnl1* remains largely unknown.

We have now analyzed the expression pattern of *Vsnl1* mRNA and protein during mouse embryogenesis, with special emphasis on its expression in the heart and vascular system.

1. Results and discussion

1.1. Vsnl1 expression in developing mouse heart and circulatory system

To address the spatial and temporal expression patterns of Vsnl1 during mouse development, we performed whole-mount *in situ* hybridization and immunolabeling of E8 to E18 embryos.