Gene Expression Patterns 11 (2011) 216–220

Contents lists available at ScienceDirect

Gene Expression Patterns

journal homepage: www.elsevier.com/locate/gep

XLMR candidate mouse gene, Zcchc12 (Sizn1) is a novel marker of Cajal–Retzius cells

Ginam Cho [a], Youngshin Lim [b], Jeffrey A. Golden [a,*]

[a] Department of Pathology and Laboratory Medicine, The Children's Hospital of Philadelphia, Philadelphia, PA 19104, USA
[b] Department of Pathology and Laboratory Medicine, University of Pennsylvania, USA

ARTICLE INFO

Article history:
Received 18 May 2010
Received in revised form 10 December 2010
Accepted 14 December 2010
Available online 21 December 2010

Keywords:
Sizn1 (Zcchc12)
Cajal–Retzius cell
BMP
Pallial–subpallial boundary
Cortical hem
Septum
Reln

ABSTRACT

Sizn1 (Zcchc12) is a transcriptional co-activator that positively modulates bone morphogenic protein (BMP) signaling through its interaction with Smad family members and CBP. We have demonstrated a role for Sizn1 in basal forebrain cholinergic neuron specific gene expression. Furthermore, mutations in *SIZN1* have been associated with X-linked mental retardation. Given the defined role of *SIZN1* in mental retardation, knowing its complete forebrain expression pattern is essential to further elucidating its role in cognition. To better define the dynamic expression pattern of *Sizn1* during forebrain development, we investigated its expression in mouse brain development from embryonic day 8.0 (E8.0) to adult. We found that *Sizn1* is primarily restricted to the ventral forebrain including the medial ganglionic eminence, the septum, amygdala, and striatum. In addition, *Sizn1* expression is detected in the cortical hem and pallial–subpallial boundary (PSB; anti-hem); both sources of Cajal–Retzius cells. *Sizn1* expression in the dorsal forebrain is restricted to a subset of cells in the marginal zone that also express *Reln*, indicative of Cajal–Retzius cells. These data provide novel information on brain regions and cell types that express Sizn1, facilitating further investigations into the function of Sizn1 in both development and the pathogenesis of mental retardation.

Over the past decade mutations in numerous genes have been associated with mental retardation, yet the pathogenesis remains poorly understood in all but a few disorders. *SIZN1* (*ZCCHC12*) is one example of such a gene that, when mutated, results in mental retardation in males (Cho et al., 2008a). Biochemical studies indicate that Sizn1 is a positive modulator of BMP signaling and is necessary for normal basal forebrain cholinergic neuron gene expression (Cho et al., 2008b).

Basal forebrain cholinergic neurons are known to be the major cholinergic input to the cerebral cortex and hippocampus (Bigl et al., 1982; Mesulam et al., 1983a,b; Woolf et al., 1983, 1984). Loss of this input correlates with cognitive decline in Alzheimer's disease, implicating an important role of basal forebrain cholinergic neurons in cognition (Baxter and Chiba, 1999; Granholm et al., 2000). In addition, the embryonic septum is not only the origin for many basal forebrain cholinergic neurons, but also a subset of Cajal–Retzius (CR) cell that expresses Reln and are important for normal neocortex lamination. Cajal–Retzius neurons are born from several sites including the septum, the pallial–subpallial boundary, and the cortical hem (Bielle et al., 2005; Meyer et al., 2002; Shinozaki et al., 2002; Takiguchi-Hayashi et al., 2004). From these sites they migrate to the marginal zone to form layer I of the cerebral cortex.

We have hypothesized that mutations in *SIZN1* lead to basal forebrain cholinergic neuron deficiencies and therefore intellectual disabilities. Knowing the expression pattern for *SIZN1/Sizn1* through development is necessary to gain an understanding of the pathogenesis of *SIZN1*-associated mental retardation. Therefore, we undertook a series of studies to determine the cell types that express Sizn1 within and beyond the basal forebrain cholinergic neurons. Using both *in situ* hybridization and immunohistochemistry, we found Sizn1 is expressed in ventral forebrain cell populations in addition to the cholinergic neuron. Furthermore, we find Sizn1 is expressed by Cajal–Retzius neurons of the dorsal forebrain, beginning in their progenitor zones. These data implicate additional sites where Sizn1 is potentially functioning and contributing to a mental retardation phenotype.

1. Results and discussion

1.1. Genomic structure of Sizn1 and sequence comparison

Sizn1 is located on the X-chromosome (Cho et al., 2008b) and is composed of 4 exons with the entire coding sequence residing in

* Corresponding author. Address: Abramson Research Center, Room 516F, 3400 Civic Center Blvd., Philadelphia, PA 19104, USA. Tel.: +1 215 590 4307; fax: +1 215 590 3709.
E-mail address: goldenj@mail.med.upenn.edu (J.A. Golden).

1567-133X/$ - see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.gep.2010.12.005

the fourth exon. Sequence comparison using the blast algorithm at Ensembl and UCSC genome browser shows that mammalian orthologs are present only in mammals including humans, cows, chimpanzee, rat and mice.

1.2. Sizn1 transcripts are detected in a dynamic pattern

Sizn1 mRNA expression was first analyzed by whole-mount *in situ* hybridization. At E9.5, *Sizn1* was detected mainly in the rostral neural tube and in primordial germ cells (Fig. 1A). In the brain, *Sizn1* was detected in the dorsal midline of the telencephalon in the same region where Bmp and Wnt family members are also expressed (Fig. 1A and B) (Grove et al., 1998; Shimogori et al., 2004). This dorsal midline expression was limited to the telencephalon; no extension to the dorsal midline of the midbrain or hindbrain was observed (Fig. 1A). In the hindbrain (rhombencephalon), Sizn1 expression was found along the ventral midline anteriorly (Fig. 1A and C). Outside the CNS (central nervous system), *Sizn1* expression was detected in the migrating primordial germ cells (Fig. 1D), which are precursors of germ cells known to originate from the primitive streak before E7.0. These cells migrate to the hindgut to settle in the genital ridges and form adult germ cells (Tres et al., 2004). Expression of Sizn1 in testis occurs at E14.5 (data not shown) and in adult as described previously (Cho et al., 2008b).

By E12.5, *Sizn1* was no longer detected along the expanded dorsal midline of the telencephalon (Fig. 1E and F), however, expression in the cortical hem (hippocampus) was detected (see Fig. 2C, G and H). In addition, it was detected in the lateral olfactory bulb (LOT), amygdala and pallial–subpallial boundary (PSB) (Fig. 1E). Prominent expression was found in the septum as previously described (Cho et al., 2008b) and zona limitans intrathalamica (ZLI) (Fig. 1F). ZLI expression was confirmed by comparing expression with the known ZLI marker, *Shh* (Fig. 1G) (Puelles and Rubenstein, 2003). In the midbrain (mesencephalon) and hindbrain (rhombencephalona), Sizn1 is mostly restricted ventrally, although the expressions extended more laterally when compared to the earlier stages (Fig. 1F and C).

At E14.5, *Sizn1* expression in the brain primarily mirrors that seen at E12.5. Its expression in the telencephalon was mainly restricted to ventral areas including the striatum, amygdala (Fig. 1H and I) and septum (Fig. 1J), the ventral diencephalon and the hypothalamic nuclei (Fig. 1H and J). Interestingly, expression in the midbrain spans the entire neural tube from ventral to dorsal (Fig. 1I and J). Although previous reports suggested that Sizn1

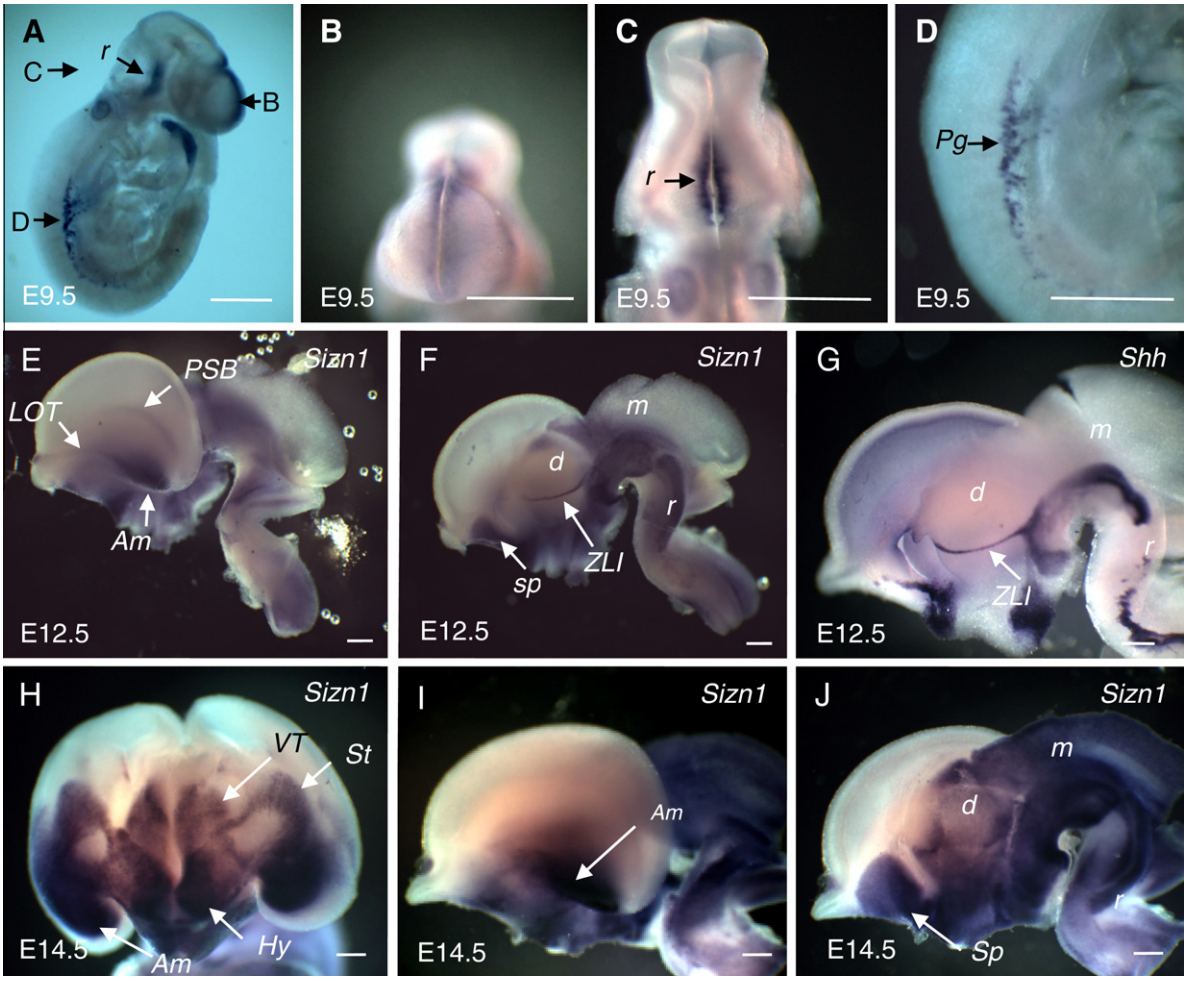

Fig. 1. *In situ* hybridization for *Sizn1* at E9.5, E12.5 and E14.5 whole mouse embryos. In E9.5 stage, *Sizn1* is expressed in dorsal midline of the forebrain (A and B), the ventral rhombencephalon (A and C) and in primodial germ cells (A and D). In E12.5 stage, the expression of *Sizn1* is now restricted to the ventral neural tube from the telencephalon through the rhombencephalon (E, lateral view and F, medial view). Expression is found in the lateral olfactory tract (LOT), pallium-subpallium boundary (PSB), amygdala, septum and zona limitans intrathalamica (ZLI). (G) The ZLI expression was confirmed by comparison with *Shh-in situ* hybridization, which is known to be expressed in the ZLI (Puelles and Rubenstein, 2003). While the pattern of E14.5 is generally similar to that at E12.5, there has been considerable expansion of the expression domains. In a mid coronal section of the whole brain (E14.5) shows strong expression in the amygdala, hypothalamus, ventral thalamus and striatum (H). A lateral (I) and medial (J) mid-sagittal view also shows extensive expression nearly circumferentially in the mesencephalon and rhombencephalon. *Am* = amygdala, *d* = diencepahlon, *Hy* = hypothalamus, *m* = mesencephalon, *Pg* = primodial germ cell, *r* = rhombencephalon, *Sp* = Septum, *St* = striatum and *VT* = ventral thalamus. Scale bar is adjusted to 500 μm in each image.