Lysine-specific demethylase 1 expression in zebrafish during the early stages of neuronal development

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Abstract
Lysine-specific demethylase 1 (Lsd1) is associated with transcriptional coregulation via the modulation of histone methylation. The expression pattern and function of zebrafish Lsd1 has not, however, been studied. Here, we describe the pattern of zebrafish Lsd1 expression during different development stages. In the zebrafish embryo, lsd1 mRNA was present during the early cleavage stage, indicating that maternally derived Lsd1 protein is involved in embryonic patterning. During embryogenesis from 0 to 48 hours post-fertilization (hpf), the expression of lsd1 mRNA in the embryo was ubiquitous before 12 hpf and then became restricted to the anterior of the embryo (particularly in the brain) from 24 hpf to 72 hpf. Inhibition of Lsd1 activity (by exposure to tranylcypromine) or knockdown of lsd1 expression (by morpholino antisense oligonucleotide injection) led to the loss of cells in the brain and to a dramatic downregulation of neural genes, including gad65, gad75, and reelin, but not hey1. These findings indicate an important role of Lsd1 during nervous system development in zebrafish.

Key Words
zebrafish; lysine-specific demethylase; morpholino; tranylcypromine; nerve cells; embryonic development; histone methylation; histone demethylase; brain; neural regeneration

Research Highlights
(1) We utilized a zebrafish model to study Lsd1 expression and function.
(2) Inhibition of zebrafish Lsd1 activity led to significant cell loss in the brain.
(3) Inhibition of Lsd1 activity led to the downregulation of neural genes, such as gad65, gad75 and reelin, but not hey1.

Abbreviations
LSD, lysine specific demethylase; CNS, central nervous system; MO, morpholino antisense oligonucleotides
INTRODUCTION

Lysine specific demethylase 1 (Lsd1, also known as BHC110 or AOF2) is a flavin-dependent histone demethylase that specifically removes methyl groups from Lys4 of histone H3 (H3K4), which is important for a wide range of biological processes, such as development, differentiation, cancer, and neurological disorders. The structure and function of Lsd1 is conserved from yeast to humans. Shi has described the discovery of a new family member – Lsd2 (AOF1). Lsd1 is comprised of an N-terminal SWIRM (Swi3p, Rsc8p and Moira) domain and a C-terminal flavin domain, which shows homology to members of the amine oxidase family. Lsd1 has been found in association with CoREST and histone deacetylases 1 and 2. In the central nervous system (CNS), chromatin integrates a number of converging signaling pathways, leading to short- and long-term changes in gene expression that are crucial for neuronal commitment and terminal maturation, as well as for neuroplasticity throughout the life. Although a variety of ubiquitously expressed chromatin-remodeling complexes support tissue-specific transcription factors, neurorestricted chromatin-remodeling factors have only recently been identified. Using standard gene targeting technology in mouse embryonic stem cells, Lsd1 knockout causes death in prenatal and perinatal stages. Despite the sudden cardiac death phenotype, the lsd1 knockout strategy resulted in a major effect on the development of the pituitary and neural systems at a very early stage.

Over the past decade, the zebrafish model has come to the forefront of biological research; this model system has allowed the elucidation of a plethora of fundamental developmental processes. Apart from being a vertebrate with organs and tissues, such as a brain and spinal cord, that have conserved organization, the zebrafish system bridges the gap between fruit fly/worm and mouse/human genetics, making it feasible to address issues of early development, organ formation, integrative physiology, pharmacology, and complex disease. In the absence of blood circulation, zebrafish receive some oxygen by passive diffusion and are able to survive and develop in a relatively normal fashion for several days, thereby allowing a detailed analysis of animals with severe cardiovascular defects.

Although an increasing number of histone demethylases have been identified and biochemically characterized, their biological functions, particularly in the context of an animal model, are not well understood. There is some information regarding the expression and function of Lsd1 in the mouse brain; however, only limited studies have been performed on zebrafish. Thus, we used zebrafish as a model to study the functions and related mechanisms of Lsd1. Here, we report on the Lsd1 expression pattern during the early developmental stages and further demonstrate a neuronal phenotype. Our results also suggest that zebrafish is a promising model for the detection of nerve disorders.

RESULTS

**lsd1 mRNA expression during zebrafish development**

To localize lsd1 transcripts during zebrafish development, we performed whole-mount RNA in situ hybridization. At early stages (12 hpf, hours post-fertilization), lsd1 mRNA was localized throughout the whole embryo. At late somitogenesis (24 hpf), lsd1 expression was observed in the head and spinal cord (Figure 1A). By the late pharyngula stages (48 hpf), lsd1 expression was restricted to the anterior region. At the early larval stages (72 hpf), lsd1 was expressed in a more refined region, but in a similar pattern to that at 48 hpf. Expression of lsd1 was present in the brain (such as in the neural fields in the diencephalon and dorsal hindbrain; Figures 1A, C) and spinal cord. Spinal cord expression occupied three planes along the dorsoventral axis, presumably the motor neurons and interneurons.

Furthermore, lsd1 transcripts showed strong expression during very early stages. At the one cell stage, strong lsd1 expression was distributed throughout the embryo (Figure 1A).

To determine if the transcript levels observed by whole-mount RNA in situ hybridization on staged zebrafish embryos were consistent with the hypothesized expression during neurogenesis, we performed semi-quantitative reverse transcription (RT)-PCR analysis on 12 to 72 hpf embryos (Figure 1B) and found that transcripts were present at each stage investigated.

**Exposure to PCPA (tranylcypromine) or morpholino antisense oligonucleotide (MO) injection inhibited Lsd1 histone demethylase activity**

The zebrafish lsd1 gene contains 21 exons, encodes 833 amino acids, and is located on chromosome 17. To study the effects of lsd1 downregulation on embryonic development, morpholino antisense oligonucleotides (lsd1 MO) were designed to block splicing of exon 11 of lsd1, which plays a crucial role in histone demethylation.
Using RT-PCR, we found that injection of the \textit{lsd1} MO into early zebrafish embryos resulted in the production of aberrantly spliced messages (Figure 2A). Sequence analysis of the corresponding cDNAs revealed that deletion of the targeted exon (exon 11) was caused by the \textit{lsd1} MO, and that normal \textit{lsd1} was partially downregulated. The effect of morpholinos was also tested by western blot analysis (Figures 2B, C). Western blotting showed that the H3K4 demethylation level was increased significantly in the MO group as well as in the PCPA group compared to the controls. Therefore, a loss-of-function \textit{lsd1} zebrafish can be obtained by \textit{lsd1} MO or PCPA treatment.

\textit{lsd1} was expressed in the CNS region in early developmental stages. Therefore, we addressed the question of whether a neuronal phenotype occurs when Lsd1 activity was inhibited.

\textbf{The expression of \textit{gad65}, \textit{gad75}, and \textit{reelin} decreased when Lsd1 activity was inhibited}

After injection of \textit{lsd1} MO, histological examination revealed that a mass of cells were absent in the brain CNS region (particularly in the tectum) and the structure of the trabecula was dramatically altered (Figure 3B). Further examination by RT-PCR showed the expression of several neuronal markers, including \textit{gad65}, \textit{gad75}, \textit{reelin}, and \textit{hey1}. Strikingly, we found that injection of Lsd1 MO or exposure to PCPA significantly inhibited \textit{gad65}, \textit{gad75}, and \textit{reelin} expression ($P < 0.05$) at 24 hpf. However, the expression of \textit{hey1} showed a slight increase in the MO group (Figure 3A). These data indicate that the downregulated expression of \textit{gad65}, \textit{gad75}, and \textit{reelin} may be connected with impaired nerve cells.
Zebrafish models for the study of neurogenic disorders

In recent years, zebrafish have been used to study neurodegenerative disorders, such as Alzheimer’s disease, Parkinson’s disease, and Huntington’s disease, with an emphasis on studies of degenerative neurological diseases.[8, 16] More recently, studies have examined the tau protein in zebrafish, which may contribute to understanding Alzheimer’s disease in human.[18] Research into Parkinson’s disease has also used zebrafish as a model.[17-19] Another CNS disorder that has been studied recently using zebrafish is epilepsy as clonus-like seizures can be induced by convulsant agents.[14, 20-21] This has permitted forward mutagenesis screens and the isolation of seizure-resistant mutants.[22]

These recent developments with the new model (especially with transgenic zebrafish) open the possibility of further screens to dissect the molecular mechanisms and discover novel therapeutics for neurodegenerative disorders.[23-24]

The characteristics of Lsd1 expression in zebrafish and mouse

The Lsd1 expression pattern was first described in mouse; the processing of the \(lsd1\) transcript undergoes a dynamic histone demethylase activity modulation within the perinatal window when fundamental processes take place, such as phylopodia formation, neurite sprouting, and the expression of the first synaptogenic markers. Transcriptional profiling of the developing rat brain revealed that the most dramatic changes in gene expression occur post-partum[25], underscoring the relevance of early postnatal life as a critical phase of neural organization and differentiation. Mouse \(lsd1\) transcripts are seen from embryonic day 10.5 until 2 weeks post-partum[8], but our data show that maternal transcripts are present in zebrafish. Transcriptional profiling of the developing rat brain reveals that the most dramatic regional changes in gene expression occur post-partum[8]. However, \(lsd1\) \(-/-\) mice die within 22 days and more extensive work cannot be done with this model. Thus, other in vivo models should be employed.

The present study also demonstrates an important role for \(lsd1\) in embryonic neuronal development[26-27]. In zebrafish, \(lsd1\) is expressed throughout the developing stages: from the one cell embryo the expression pattern becomes restricted to the anterior of the embryo and is particularly enriched at the neural structures as embryonic development progresses. Zebrafish neurogenesis begins at the early tail bud stages (9 hpf), reaches high levels 24–48 hpf, and progresses 72 hpf. The timing of peak \(insm1a\) and \(insm1b\) expression coincides precisely with periods of zebrafish neurogenesis. The \(lsd1\) expression in one cell and two cells stages suggests that \(lsd1\) transcripts appear to be maternally contributed and are important for the original
polarization.

Maternally derived Lsd1 and lsd1 mRNA are present during early cleavage stages in the zebrafish embryo. lsd1 mRNA is detectable in the 1–4 cell-stage embryo, before the start of zygotic gene transcription. Later during embryogenesis, lsd1 mRNA is expressed in the majority of the embryo (especially the CNS). Maternally derived lsd1 is most likely the substrate for the lsd1 transporter at these stages of development because liver, which can synthesize Lsd1 endogenously, does not become functional in zebrafish embryos until 2 days after fertilization. In our study, splice morpholinos are used to block translation, but almost all new zygotic transcription occurs only after the midblastula transition, and the splice morpholin is very unlikely to affect demethylation in 1–2 cells. Thus, we used PCPA to inhibit the H3K4 (2m) demethylase action of Lsd1.

Lsd1 functions in neurogenetic development

Lsd1 downregulation causes a variety of disorders in many tissues, especially the nervous system[22, 25, 28]. The functional characteristics of zebrafish Lsd1 are very similar to those of mammalian Lsd1s, including histone demethylase activity. In zebrafish development, the spatial and temporal patterns of neural induction and axonal tract formation were assessed before 48 hpf. To test whether a special phenotype was induced by neural damage at an early stage, we examined the expression of several neuronal markers, including gad65, gad75, reelin, and hey1, following knockdown of lsd1 expression at 24 hpf.

Reelin is an extracellular matrix protein that is synthesized and secreted from cortical GABAergic interneurons[29-30]. Reelin surrounds apical and basal dendritic spines of pyramidal cortical neurons[31]. Reelin plays a defined role not only in prenatal CNS development[32-33], but also in the adult brain by modulating cortical pyramidal neuron dendritic spine expression density, the branching of dendrites, and the expression of long-term potentiation[31, 34]. It is likely that reelin has a role in regulating the event-related increase in protein synthesis that is mediated by the dendritic translation of cytosolic mRNAs[33]. Gad65 exists primarily as an apoenzyme under normal conditions, whereas Gad67 exists as a holoenzyme. Despite extensive study, little is known about Gad65 and Gad67.

Interestingly, the downregulation of Gad65, as well as Gad67, is likely to be responsible for working memory impairment, and the downregulation of reelin expression in neocortex and hippocampus may be important in mediating the downregulation of pyramidal neuron dendritic branching and spine expression and in the neuropil hypoplasia typical of schizophrenia patients[35-37]. The histone demethylase activity of Lsd1 may have a regulatory role in the expression of these three target genes (reelin, gad65 and gad67). Therefore, Lsd1 deficiency in the CNS may be involved in human schizophrenia.

Hey1 is implicated as a potential regulator of cell differentiation and proliferation through the Notch pathway[38-39]. However, the expression of hey1 remains unchanged in the MO group in our study. Therefore, our result suggests that the Notch signaling pathway is not likely to be involved in the regulation of the loss-of-function Lsd1 zebrafish.

In summary, in the zebrafish embryo, lsd1 mRNA is present during early cleavage stages, indicating that maternally derived Lsd1 protein is involved with embryonic patterning. During embryogenesis (from 0 to 48 hpf), the expression of lsd1 mRNA in the embryo is ubiquitous before 12 hpf and is more restricted to anterior regions (particularly the brain) from 24 to 72 hpf, which is consistent with neuronal differentiation. Inhibition of Lsd1 activity (using tranylcypromine, PCPA) or knockdown of lsd1 expression (using siRNA morpholino) leads to a loss of cells in the brain region and dramatically downregulated neural genes, including gad65, gad75, and reelin, but not hey1. These results provide insight into the expression pattern and molecular function of the lower vertebrate lsd1 gene.

MATERIALS AND METHODS

Design
A controlled, observational, in vivo study.

Time and setting
Experiments were conducted at the Immunology Laboratory, Nantong University, China from January to July 2011.

Materials
Animals
Wild-type (AB* strain) zebrafish stocks were obtained from the International Zebrafish Research Center (WT group). Embryos were obtained from natural spawning of wild-type adults. Zebrafish were raised, maintained, and staged as previously described[9].
Drugs
Lsd1 inhibitors, trans-2-phenylcyclopropylamine (referred to as PCPA hereafter; also known as tranylcypromine and parnate; Sigma, USA), were dissolved in ddH2O at stock concentrations of 100 mM, and then diluted in embryo media to a final concentration of 40 μM. Control embryos were wild-type zebrafish. All embryos were incubated at 28.5°C[15].

Methods
Whole-mount RNA in situ hybridization
Plasmids encoding zebrafish lsd1 (3'-UTR region) were kindly provided by Nantong University, China. Whole-mount RNA in situ hybridization using digoxigenin-labeled antisense RNA probes was performed using standard methods as previously described[14]. Briefly, digoxigenin-labeled antisense RNA probes were produced using a digoxigenin-RNA labeling kit (Roche, Mannheim, Germany) following the manufacturers’ instructions. Hybridization and detection with an anti-digoxigenin antibody coupled to alkaline phosphatase was performed with fixed zebrafish embryos.

Western blot assay
Embryos (24 hpf) were completely deyolked in Ginzburg Fish Ringer buffer (55 mM NaCl, 1.8 mM KCl, 1.25 mM NaHCO₃) and washed with PBS. The embryos were collected by centrifugation and lysed with 2 × Laemmli’s buffer for 30 minutes followed by centrifugation to remove the debris. After separation by 12.5% sodium dodecylsulfate polyacrylamide gel electrophoresis, the proteins were blotted onto a nitrocellulose filter and hybridized with rabbit polyclonal anti-H3K4 demethylation (H3K4 (2m)) antibody (1:1 000; Abcam, Cambridge, MA, USA).

Morpholino oligonucleotide injection
Morpholinos were obtained from Gene Tools (Philomath, OR, USA). For lsd1, the splice morpholino (exon 11) contained 25 bases. For control, a lsd1 mismatch morpholino (control MO, CON group) that included five mispaired bases was used. The sequences of lsd1 MO and CON (mis-match MO) were GGG ACC TCT CTA CTA CGA CAA GAG-3' and GGc ACC TCT CTA CTA CGA CAA GAG-3', respectively. 0.05 μL lsd1 MO at 15.0 μg/mL (1 ng per embryo) was injected into the blastomere of one-cell or two-cell stage embryos (microinjector from Narishige, Japan). Control MO was injected at the same volume.

RT-PCR
Total RNA was extracted from 25–30 embryos using TRIzol Reagent (Invitrogen, Carlsbad, CA, USA) according to manufacturer’s instructions. One microgram of total RNA was used as a template in a 20 mL RT-PCR reaction mixture using a one-step RT-PCR kit (Qiagen, Hilden, Germany). Quantitative analysis of RT-PCR expression proction by REST soft (Stratagene, La Jolla, CA, USA). The RT-PCR conditions are as previously described[10], except for a change in annealing temperature, which depended on the Tm value of the primers. PCR primers used to detect targeted exon 11 skipping are listed in Table 1. Product concentration was quantified using gapdh as an internal reference.

| Gene    | Primer sequence            |
|---------|----------------------------|
| lsd1    | Upstream: 5'- GAA GGA GAC GGT GGT GAG-3' |
|         | Downstream: 5'-GCT ACA AAG TGA GGG ACA -3' |
| gapdh   | Upstream: 5'-ACC ACA GCT CAT CAC-3' |
|         | Downstream: 5'-TCC ACC ACC CTG TTG CTG TA-3' |
| gad65   | Upstream: 5'-TCT CCA ATC CAG CCG CTA CCT-3' |
|         | Downstream: 5'-GCA ATC CAT CCA GGG ACA CG-3' |
| gad67   | Upstream: 5'-TAG TCC ATT GGG ATG ATC ACA GCC-3' |
|         | Downstream: 5'-CAA GAC CTA CGA CAA GGG AAG-3' |
| reelin  | Upstream: 5'-AGC TGT AAC ACT GGG CTA GAT-3' |
|         | Downstream: 5'-TGT AGG ACA CTC TCT GGG TT-3' |
| hey1    | Upstream: 5'-GAA GCC CTC CCA GTC ACC-3' |
|         | Downstream: 5'-CTT AGC GGT CTC GTC TGC-3' |

Histological examinations
At 6 dpf, 20 fish from each treatment were pooled and euthanized with Tricaine (Sigma). Bodies were fixed in Dietrich’s fixative[40] and then embedded in paraffin and sectioned longitudinally along the entire dorsal-ventral axis at 4mm. Sections were stained with hematoxylin-eosin and examined under a light microscope (Olympus, Tokyo, Japan).

Photography
Stained embryos were examined with Olympus BX61 and SZX12 microscopes (Olympus, Japan), and photographed with a DP70 digital camera (Olympus). Images were processed using IMAGE PRO software (Media Cybernetics, Silver Spring, MD, USA).

Statistical analysis
All the results are expressed as mean ± SD and analyzed by SPSS 17.0 statistical software (SPSS, Chicago, IL, USA). Differences among groups were analyzed by one-way analysis of variance, and an independent sample t-test was used to analyze the comparisons of MO/PCPA and WT/control zebrafish in western blot. A P value < 0.05 was considered statistically significant.
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Conflicts of interest: None declared.

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REFERENCES

[1] Allis CD, Berger SL, Cote J, et al. New nomenclature for chromatin-modifying enzymes. Cell. 2007;131:633-636.

[2] Yang M, Culhane JC, Szewczuk LM, et al. Structural basis for the inhibition of the LSD1 histone demethylase by the antidepressant trans-2-phenylcyclopropylamine. Biochemistry. 2007;46:8058-8065.

[3] Forneris F, Binda C, Battaglioli E, et al. LSD1: oxidative chemistry for multifaceted functions in chromatin regulation. Trends Biochem Sci. 2008;33:181-189.

[4] Nottke A, Colaiacovo MP, Shi Y. Developmental roles of the histone lysine demethylases. Development. 2009;136:879-889.

[5] Shi Y. Histone lysine demethylases: emerging roles in development, physiology and disease. Nat Rev Genet. 2007;8:829-833.

[6] Shi Y, Lan F, Matson C et al. Histone demethylation mediated by the nuclear amine oxidase homolog LSD1. Cell. 2004;119:941-953.

[7] Zibetti C, Adamo A, Binda C, et al. Alternative splicing of the histone demethylase LSD1/KDM1 contributes to the modulation of neurite morphogenesis in the mammalian nervous system. J Neurosci. 2010;30:2521-2532.

[8] Wang J, Scully K, Zhu X, et al. Opposing LSD1 complexes function in developmental gene activation and repression programmes. Nature. 2007;446:882-887.

[9] Zibetti C, Adamo A, Binda C, et al. Alternative splicing of the histone demethylase LSD1/KDM1 contributes to the modulation of neurite morphogenesis in the mammalian nervous system. J Neurosci. 2010;30:2521-2532.

[10] Pyati UJ, Look AT, Hammerschmidt M. Zebrafish as a powerful vertebrate model system for in vivo studies of cell death. Semin Cancer Biol. 2007;17:154-165.

[11] Peterson RT, Shaw SY, Peterson TA, et al. Chemical suppression of a genetic mutation in a zebrafish model of aortic coarctation. Nat Biotechnol. 2004;22:595-599.

[12] Neely MN, Pfeifer JD, Caparon M. Streptococcus-zebrafish model of bacterial pathogenesis. Infect Immun. 2002;70:3904-3014.

[13] Best JD, Alderton WK. Zebrafish: An in vivo model for the study of neurological diseases. Neuropsychiatr Dis Treat. 2008;4:567-576.

[14] Kabashi E, Brustein E, Champagne N, et al. Zebrafish models for the functional genomics of neurogenetic disorders. Biochim Biophys Acta. 2011;1812:335-345.

[15] Zhang J, Tan L, Hou JY, et al. Trans-2-phenylcyclopolyamine induces nerve cells apoptosis in zebrafish mediated by depression of LSD1 activity. Brain Res Bull. 2009;80:79-84.

[16] Ingham PW. The power of the zebrafish for disease analysis. Hum Mol Genet. 2009;18:R107-112.

[17] Abeliovich A, Flint Beal M. Parkinsonism genes: culprits and clues. J Neurochem. 2006;99:1062-1072.

[18] Flinn L, Bretaud S, Lo C, et al. Zebrafish as a new animal model for movement disorders. J Neurochem. 2008;106:1991-1997.

[19] Bandmann O, Burton EA. Genetic zebrafish models of neurodegenerative diseases. Neurobiol Dis. 2010;40:58-65.

[20] Baraban SC. Emerging epilepsy models: insights from mice, flies, worms and fish. Curr Opin Neurol 2007;20:164-168.

[21] Tiedeken JA, Ramsdell JS. DDT exposure of zebrafish embryos enhances seizure susceptibility: relationship to fetal p,p'-DDE burden and domoic acid exposure of California sea lions. Environ Health Perspect. 2009;117:68-73.

[22] Baraban SC, Dinday MT, Castro PA, et al. A large-scale mutagenesis screen to identify seizure-resistant zebrafish. Epilepsia. 2007;48:1151-1157.

[23] Li YJ, Hu B. Establishment of multi-site infection model in zebrafish larvae for studying staphylococcus aureus infectious disease. J Genet Genomics. 2012;39:521-534.

[24] Li Z, Zheng W, Wang Z, et al. An inducible Myc zebrafish liver tumor model revealed conserved Myc signatures with mammalian liver tumors. Dis Model Mech. in press.

[25] Stead JD, Neal C, Meng F, et al. Transcriptional profiling of the developing rat brain reveals that the most dramatic regional differentiation in gene expression occurs postpartum. J Neurosci. 2006;26:345-353.
[26] Ceballos-Chávez M, Rivero S, García-Gutiérrez P, et al. Control of neuronal differentiation by sumoylation of BRAF35, a subunit of the LSD1-CoREST histone demethylase complex. Proc Natl Acad Sci U S A. 2012;109:8085-8090.

[27] Fuentes P, Cánovas J, Berndt FA, et al. CoREST/LSD1 control the development of pyramidal cortical neurons. Cereb Cortex. 2012;22:1431-1441.

[28] Zhang GR, Zhao H, Cao H, et al. Overexpression of either lysine-specific demethylase-1 or CLOCK, but not Co-Rest, improves long-term expression from a modified neurofilament promoter, in a helper virus-free HSV-1 vector system. Brain Res. 2012;1436:157-167.

[29] Wang X, Babayan AH, Basbaum AI, et al. Loss of the Reelin-signaling pathway differentially disrupts heat, mechanical and chemical nociceptive processing. Neuroscience. 2012;226C:441-450.

[30] Arancha BL, Inmaculada CL, Tiziana C, et al. Be-amyloid controls altered Reelin expression and processing in Alzheimer's disease. Neurobiol Dis. 2010;37:682-691.

[31] Costa E, Davis J, Grayson DR, et al. Dendritic spine hypoplasticity and downregulation of reelin and GABAergic tone in schizophrenia vulnerability. Neurobiol Dis. 2001;8:723-742.

[32] Niu S, Renfro A, Quattrocchi CC, et al. Reelin promotes hippocampal dendrite development through the VLDLR/ApoER2-Dab1 pathway. Neuron. 2004;41:71-84.

[33] Schmid RS, Jo R, Shelton S, et al. Reelin, integrin and DAB1 interactions during embryonic cerebral cortical development. Cereb Cortex. 2005;15:1632-1636.

[34] Weeber EJ, Beffert U, Jones C, et al. Reelin and ApoE receptors cooperate to enhance hippocampal synaptic plasticity and learning. J Biol Chem. 2002;277:39944-39952.

[35] Kvajo M, McKellar H, Gogos JA, et al. Avoiding mouse traps in schizophrenia genetics: lessons and promises from current and emerging mouse models. Neuroscience. 2012;211:136-164.

[36] Fatemi SH, Earle JA, McMenomy T. Reduction in Reelin immunoreactivity in hippocampus of subjects with schizophrenia, bipolar disorder and major depression. Mol Psychiatry. 2000;5:654-663,571.

[37] Spencer KM, Nestor PG, Perlmutter R, et al. Neural synchrony indexes disordered perception and cognition in schizophrenia. Proc Natl Acad Sci U S A. 2004;101:17288-17293.

[38] O'Brien LL, Grimaldi M, Kostun Z, et al. Wt1a, Foxc1a, and the Notch mediator Rbpj physically interact and regulate the formation of podocytes in zebrafish. Dev Biol. 2011;358:318-330.

[39] Zhang L, Widau RC, Herring BP, et al. Delta-like 1-Lysine613 regulates notch signaling. Biochim Biophys Acta. 2011;1813:2036-2043.

[40] Westerfield M. The Zebrafish Book. 5th ed. Eugene, OR: University of Oregon. 2007.

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