The schizophrenia-associated missense variant rs13107325 regulates dendritic spine density

Shiwu Li1,10, Changguo Ma1,10, Yifan Li1,10, Rui Chen1,2,10, Yixing Liu1,10, Li Pear Wan2,3,10, Qiu Xia Xiong4, Chuang Wang10, Yongxia Huo1, Xinglun Dang1,2, Yongfeng Yang10, Luxian Lv6, Xi Chen7, Nengyin Sheng4,5, Wenqiang Li6,10 and Xiong-Jian Luo1✉

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The schizophrenia-associated missense variant rs13107325 (C/T) in SLC39A8 consistently showed robust association with schizophrenia in recent genome-wide association studies (GWASs), suggesting the potential pathogenicity of this non-synonymous risk variant. Nevertheless, how this missense variant confers schizophrenia risk remains unknown. Here we constructed a knock-in mouse model (by introducing a threonine at the 393th amino acid of mouse SLC39A8 (SLC39A8-p.393T), which corresponds to rs13107325 (p.Ala391Thr) of human SLC39A8) to explore the potential roles and biological effects of this missense variant in schizophrenia pathogenesis. We assessed multiple phenotypes and traits (associated with rs13107325) of the knock-in mice, including body and brain weight, concentrations of metal ions (including cadmium, zinc, manganese, and iron) transported by SLC39A8, blood lipids, proliferation and migration of neural stem cells (NSCs), cortical development, behaviors and cognition, transcriptome, dendritic spine density, and synaptic transmission. Many of the tested phenotypes did not show differences in SLC39A8-p.393T knock-in and wild-type mice. However, we found that zinc concentration in brain and blood of SLC39A8-p.393T knock-in mice was dysregulated compared with wild-types, validating the functionality of rs13107325. Further analysis indicated that cortical dendritic spine density of the SLC39A8-p.393T knock-in mice was significantly decreased compared with wild-types, indicating the important role of SLC39A8-p.393T in dendritic spine morphogenesis. These results indicated that SLC39A8-p.393T knock-in resulted in decreased dendritic spine density, thus mimicking the dendritic spine pathology observed in schizophrenia. Our study indicates that rs13107325 might confer schizophrenia risk by regulating zinc concentration and dendritic spine density, a featured characteristic that was frequently reported to be decreased in schizophrenia.

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INTRODUCTION

Recent large-scale genome-wide association studies (GWASs) have identified hundreds of risk variants for schizophrenia [1–6]. However, elucidating the roles of the identified risk variants in schizophrenia pathogenesis remains a major challenge. Risk variants in coding region play crucial roles in disease pathology [7–13], thus providing critical opportunities to translate the genetic findings into disease mechanism and clinical diagnosis and treatment [14]. Among the schizophrenia GWAS hits, a non-synonymous single nucleotide polymorphism (SNP) rs13107325 (C/T), which encodes SLC39A8 p.Ala391Thr, showed robust association with schizophrenia in schizophrenia GWASs. In 2012, Carrera et al. reported significant association between rs13107325 and schizophrenia [15]. In 2014, Schizophrenia Working Group of the Psychiatric Genomics Consortium (PGC2) validated the strong association between rs13107325 ($P = 1.54 \times 10^{-12}$) and schizophrenia [5]. In 2018, Pardinas et al. further provided strong genetic evidence for the association between rs13107325 and schizophrenia ($P = 1.19 \times 10^{-11}$) [16]. Of note, all of these genetic studies reported the consistent risk allele (i.e., T allele) of rs13107325, indicating that rs13107325 is a true risk variant for schizophrenia.

In addition to strong association between rs13107325 and diverse human traits and diseases [17, 18], another intriguing finding is the potential pathogenicity of this missense variant. rs13107325 is located in the eighth exon of SLC39A8, which encodes solute carrier family 39 member 8 (ZIP8), a transporter that is responsible for import (uptake) of metal ions cadmium, zinc, manganese, and iron [19, 20]. First, we previously showed

1Key Laboratory of Animal Models and Human Disease Mechanisms of the Chinese Academy of Sciences, Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming, Yunnan 650223, China. 2Kunming College of Life Science, University of Chinese Academy of Sciences, Kunming, Yunnan 650204, China. 3State Key Laboratory of Genetic and Resources, Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming, Yunnan 650223, China. 4Department of Clinical Laboratory, the First Affiliated Hospital of Kunming Medical University, Kunming, Yunnan 650032, China. 5Department of Pharmacology, and Provincial Key Laboratory of Pathophysiology in Ningbo University School of Medicine, Ningbo, Zhejiang 315211, China. 6Henan Mental Hospital, the Second Affiliated Hospital of Xinxiang Medical University, Xinxiang, Henan 453002, China. 7First Department of Neurosurgery, The Second Affiliated Hospital of Kunming Medical University, Kunming, Yunnan 650101, China. 8Zhongda Hospital, School of Life Sciences and Technology, Advanced Institute for Life and Health, Southeast University, Nanjing, Jiangsu 210096, China. 9Department of Neurology, Affiliated Zhongda Hospital, Southeast University, Nanjing, Jiangsu 210096, China. 10These authors contributed equally: Shiwu Li, Changguo Ma, Yifan Li, Rui Chen, Yixing Liu, Li Pear Wan.

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that rs13107325 is located in an evolutionary highly conserved coding region, strongly suggesting the functional constraint (functional conservation) of this missense variant [21]. Second, amino sequence analysis showed that the ancestral allele (i.e., C) encodes alanine (Ala) at p391, while the derived (risk) allele T encodes threonine (Thr). Ala is a hydrophobic amino acid, whereas Thr is a hydrophilic amino acid. The physicochemical property difference of Ala and Thr suggests the functionality of this variant. Third, functional annotation using the Combined Annotation Dependent Depletion (CADD) [22–24] indicated that rs13107325 had a CADD score of 34, strongly implying the deleteriousness of rs13107325. Fourth, functional studies supported that rs13107325 is a functional SNP [25]. Zhang et al. showed that rs13107325 affects cellular cadmium accumulation and toxicity [25]. Nakata et al. demonstrated the functionality of rs13107325 in Crohn’s disease by using a knock-in mouse model. They found that SLC39A8-p393T knock-in mice exhibited manganese (Mn) deficiency and increased sensitivity to epithelial injury and pathological inflammation in the colon [26]. Sunuwar et al. also reported that SLC39A8-p393T knock-in mice exhibited abnormal tissue Mn homeostasis and Mn-dependent glycosyltransferase activity in the same year [27]. These lines of evidence support that rs13107325 is a potential pathogenic variant. However, the role and potential pathophysiological mechanisms of this missense variant in schizophrenia remain unknown.

Considering the strong association between rs13107325 and schizophrenia, and the reported functional consequences of this missense variant in cadmium accumulation and Crohn’s disease [25, 26], we hypothesized that rs13107325 may also be a probable pathogenic missense variant for schizophrenia. First, we detailed the association signals (associations with schizophrenia) of the genomic region surrounding rs13107325. Among the risk variants (±250 kb from rs13107325) surrounding rs13107325, rs13107325 showed the most significant association with schizophrenia (Supplementary Figure 1). Of note, only four SNPs are in linkage disequilibrium (LD) with rs13107325 ($r^2 > 0.6$), and the association $P$ values of these SNPs were less than rs13107325. Besides, the Schizophrenia Working Group of the Psychiatric Genomics Consortium identified a credible causal set of SNPs for each of the schizophrenia risk loci, and they reported that association signals of 10 loci were credibly attributable to a known exonic missense variant [5]. Among the 10 missense variants, rs13107325 showed the most significant association with schizophrenia, strongly suggest that rs13107325 is a pathogenic variant for schizophrenia. These convergent lines of evidence support the pathogenicity of this missense variant in schizophrenia. Nevertheless, currently we know little about the role and potential pathophysiological mechanisms of this missense variant in schizophrenia. Clearly, functional characterization and biological studies of this missense SNP will not only provide important insights into the pathogenesis of schizophrenia, but also help to identify potential therapeutic targets for schizophrenia. Interestingly, the risk allele of rs13107325 only appears in European (8%) and American (5%) populations, but not exists in Asian and African populations [28–30]. Our study supports that rs13107325 is a potential pathogenic variant for schizophrenia and indicates that rs13107325 might confer schizophrenia risk by affecting density of dendritic spines.

**Materials and Methods**

**Generation of SLC39A8-p393T knock-in mice**

Detailed information about generation of SLC39A8-p393T knock-in mice (C57BL/6J background) is provided in Supplementary methods.

**Concentration measurements of cadmium, zinc, manganese, and iron**

The cerebral cortices of wild-type (SLC39A8-p.393A) and knock-in (SLC39A8-p.393T) mice were isolated (on ice) from 8-week-old mice brains. Detailed information about concentration measurements of cadmium, zinc, manganese, and iron are provided in Supplementary methods.

**Measurements of blood lipids and ions**

The blood samples were obtained from eyeballs of 8-week-old wild-type (SLC39A8-p.393A) and knock-in (SLC39A8-p.393T) mice, then placed into an anticoagulant tube and centrifugated at 1000 rpm for 10 min. The supernatant serum was used to measure the concentrations of glucose, cholesterol, triglycerides, high/low-density lipoprotein, and zinc and calcium ions using cobas® 8000 modular analyzer series (Roche) (with default parameters) at the First Affiliated Hospital of Kunming Medical University with double-blinded. A total of 20 mice were tested (wild-type: $n = 9$; knock-in: $n = 11$).

**Proliferation and migration assays**

5-ethyl-2’-deoxyuridine (EdU) was intraperitoneally injected into the pregnant heterozygous-type mice at embryonic day 13.5 (E13.5) with a dose of 50 mg/kg. Two hours post injection, brains were dissected for proliferation assays. For migration assay, 7 days post injection, brains were harvested for subsequence assays (Supplementary Figure 4A). Detailed information about proliferation and migration assays are provided in Supplementary methods.

**Cortical development evaluation**

Brains from P0 wild-type (SLC39A8-p.393A) and knock-in (SLC39A8-p.393T) mice were fixed with 4% paraformaldehyde (PFA), dehydrated by 30% sucrose, and then embedded into Tissue-Tek O.C.T. compound (Cat. No: 4583, SAKURA) at −80 °C. Coronal sections with a thickness of 10 μm were prepared by freezing microtome (Thermo Scientific, CryoStar NX50, America) at −22 °C. Coronal sections from the same cortical regions of wild-type and knock-in mice were selected to perform immunohistochemical staining. Four cortical layer-specific markers (TR1, CITP2, SOX2, SATB2) were used as previous paper to label the primary somatosensory cortex (31, 32). The primary antibodies used were rabbit anti-TR1 (Cat. No: ab31211, Abcam), rat anti-CITP2 (Cat. No: ab18446, Abcam), mouse anti-SOX2 (Cat. No: GB14149, Servicebio), rabbit anti-SATB2 (Cat. No: GB111449, Servicebio). The secondary antibodies were anti-rabbit 488 (Cat. No: GB25303, Servicebio), anti-rat Cy3 (Cat. No: GB21302, Servicebio), anti-mouse 488 (Cat. No: GB25301, Servicebio), and anti-rabbit Cy3 (Cat. No: GB21303, Servicebio). The positive cells (TR1+, CITP2+, SOX2+, SATB2+) and DAPI were counted by using Image J software (https://imagej.nih.gov/ij/) and the mouse genotypes were validated by Sanger sequencing. Coronal sections were photographed by laser scanning confocal microscope (FV1000, OLYMPUS) with 40x under the oil lens. A total of 48 photographs from 6 mice (knock-in: $n = 3$; wild-type: $n = 3$) were used for evaluating the cortical development whether affected by SLC39A8-p.393T.
Behavioral and cognitive analysis

All behavioral experiments were performed using adult male (ages: 8–12 weeks; weights: 18–26 g) wild-type (SLC39A8-p.393A) and knock-in (SLC39A8-p.393T) mice. All tested mice were placed into behavioral test house to habituate the environment at least for 1 h before behavioral tests. Detailed information about behavioral and cognitive analysis is provided in Supplementary methods.

Analysis of dendritic spine density

Golgi staining is usually used to classify and quantify dendritic spines [33, 34]. Golgi staining was performed using the FD Rapid GolgiStainTM Kit (Cat. No: PK401A, FD NeuroTechnologies), following the manufacturer’s instructions. Detailed information about dendritic spine density analysis is provided in Supplementary methods.

Electrophysiological recording on acute slices from hippocampus

Acute hippocampal slices were prepared from P18-21 wild-type (SLC39A8-p.393A) and knock-in (SLC39A8-p.393T) mice as previously described [35]. Briefly, mice were anesthetized with isoflurane and 300 μm transverse hippocampal slices were cut using a Leica VT1200 Vibratome in chilled high sucrose cutting solution, which consists of 2.5 mM KCl, 0.5 mM CaCl2, 7 mM MgCl2, 1.25 mM NaH2PO4, 25 mM NaHCO3, 7 mM D-glucose, 210 mM Sucrose, and 1.3 mM Ascorbic acid. The 300-μm-thick slices were then incubated for 30 min at 34 °C in artificial cerebrospinal fluid (ACSF) containing the following components: 119 mM NaCl, 2.5 mM KCl, 26.2 mM NaHCO3, 1 mM NaH2PO4, and 11 mM D-glucose. Then 2.5 mM CaCl2 and 1.3 mM MgSO4 were added into the ACSF, the pH of acute slices was maintained at ACSF with 95% O2/5% CO2. After 30 min incubation, the acute slices were placed at room temperature for 30–60 min and then recorded at room temperature.

For recording, individual slices were transferred to a chamber mounted in an up-right microscope (BX51W, Olympus) and perfused with ACSF (2.5 mL/min). Pyramidal neurons were identified by morphology in hippocampal CA1 region. The internal recording solution contained: 135 mM CsMeSO4, 8 mM NaCl, 10 mM HEPES, 3 mM Mg-ATP, 0.3 mM Na-GTP, and 0.1 mM spermine. The osmolarity was adjusted to 290 mOsm, and pH was buffered at 7.3–7.4. Miniature excitatory postsynaptic currents (mEPSCs) were recorded at −70 mV with 0.5 μM tetrodotoxin (TTX) in the ACSF. Whole-cell electrophysiological signals were collected with a Multiclamp 700B amplifier (Axon Instruments), filtered at 2 KHz, and digitized at 10 KHz. Custom software (IGOR Pro) was used to analyze the data offline. Statistical analyses were compared to respective controls with Mann–Whitney U test with Bonferroni correction.

RESULTS

SLC39A8-p.393T did not affect global growth of the knock-in mice

By using CRISPR-Cas9-mediated genome editing, we successfully obtained the SLC39A8-p.393T knock-in mice (Fig. 1). Compared with wild-type mice (which carried alanine at p.393), the amino acid of SLC39A8 at p.393 was changed to threonine (SLC39A8-p.393T) in knock-in mice (Fig. 1). The SLC39A8-p.393T knock-in mice thus can model the effect of risk allele of rs13107325 with wild-types at P60 stage (Fig. 2A–C), though significant body weight differences were observed at P14 to P30 stages. Further analysis indicated there was no significant difference in brain weight of the knock-in and wild-type mice (Fig. 2D). These results indicate that SLC39A8-p.393T did not affect global growth (including body and brain weight) of the knock-in mice.

SLC39A8-p.393T alters zinc concentration in brain and blood

SLC39A8 is a transporter for metal ions cadmium, zinc, manganese, and iron. To investigate if SLC39A8-p.393T affects transport of these metal ions, we measured the concentration of cadmium, zinc, manganese, and iron in brains and blood of the knock-in and wild-type mice. Cadmium, manganese, and iron did not show significant changes in brains of the knock-in and wild-type mice (Fig. 2E–G). However, concentration of zinc was significantly down-regulated in brains of the knock-in mice compared with wild-types (Fig. 2H), indicating that SLC39A8-p.393T impaired zinc uptake ability in the mouse brain. We further measured zinc concentration in blood and found that zinc concentration in blood of the knock-in mice was significantly up-regulated compared with wild-types (Fig. 2I). This result indicates that SLC39A8-p.393T impairs the zinc transport ability and leads to accumulation and elevated zinc concentration in the blood of the knock-in mice.

Considering that previous GWASs also reported significant association between rs13107325 and circulating high-density lipoprotein cholesterol (HDL-C) [36, 37], we measured the concentration of glucose, total cholesterol, triglyceride, HDL-C, and low-density lipoprotein cholesterol (LDL-C) in blood of SLC39A8-p.393T knock-in and wild-type mice. None of these tested lipids showed difference in blood of SLC39A8-p.393T knock-in and wild-type mice (Fig. 2L–O). These data indicate that SLC39A8-p.393T regulates zinc transport in brains and blood of the knock-in mice.

SLC39A8-p.393T did not affect proliferation and migration of neural stem cells

Previous studies have demonstrated the crucial roles of schizophrenia risk genes in regulating proliferation and migration of neural stem cells [38–40]. To explore if SLC39A8-p.393T knock-in mice exhibited abnormalities in proliferation and migration of NSCs in vivo, we performed EdU incorporation assays. EdU assays revealed no significant differences in proliferation and migration of NSCs in SLC39A8-p.393T knock-in and wild-type mice (Supplementary Figure 4), suggesting no obvious effect of SLC39A8-p.393T knock-in on proliferation and migration of NSCs.

Subtle effect of SLC39A8-p.393T on cortical development

Although NSCs of the SLC39A8-p.393T knock-in mice did not exhibit abnormalities in proliferation and migration, whether cortical development was affected remains unclear. We thus investigated the effect of SLC39A8-p.393T knock-in on cortical development by using four cortical layer-specific markers (TBR1, CTIP2, SOX2, SATB2) [31, 32]. We found that the ratios of TBR1+/DAPI, CTIP2+/DAPI, SOX2+/DAPI, and SATB2+/DAPI did not show significant differences between knock-in and wild-types (Fig. 3). However, when we further divided the cortex into 10 layers (as described in Moon’s study [41]), we found that the ratio of CTIP2+/DAPI was significantly higher at layer 4 and 5 in knock-in mice compared with wild-types. In addition, we also found that the ratio of TBR1+/DAPI was significantly higher at layer 4 in knock-in mice compared with wild-types (Fig. 3C, D). These results indicated the subtle effect of SLC39A8-p.393T on cortical development.

Subtle effect of SLC39A8-p.393T on transcription regulation

We next investigated the effect of SLC39A8-p.393T knock-in on gene expression in mouse brain (from P60 mice). Cortical and hippocampal tissues of SLC39A8-p.393T knock-in and wild-type mice were dissected firstly, then RNA sequencing (RNA-seq) was conducted to detect the DEGs in SLC39A8-p.393T knock-in and wild-type mice (Fig. 4A). A total of 12 DGEs were identified in cortical tissues of SLC39A8-p.393T knock-in compared with wild-type mice. Among the DEGs, 9 were down-regulated and 3 were up-regulated (Fig. 4B). In hippocampus, only 4 DEGs (Map3k6, Adipoq, Has2, and Tgfβ2) were identified (Fig. 4C). Real-time quantitative PCR (by randomly selecting 3 genes, Btg2, Zfpi189, and Has2) validated the RNA-seq results (Fig. 4D–F). Collectively, these results indicate subtle effect of SLC39A8-p.393T on transcription regulation in cortical tissues and hippocampus.
No significant behavioral and cognitive abnormalities in SLC39A8-p.393T knock-in mice compared with wild-types

To investigate if SLC39A8-p.393T affects behavioral and cognitive functions, we performed serial behavioral and cognitive tests, including open-field, light-dark transition, elevated plus maze, novel object recognition, Y-maze, 3-chambered social approach, tail suspension test. Only the total distance and distance in corner of knock-in mouse were significantly decreased compared with wild-types in the open-field test (Supplementary Figure 5B and D).

Decreased dendritic spine density in SLC39A8-p.393T knock-in mice compared with wild-types

Our above results indicated that SLC39A8-p.393T did not affect global growth (including body and brain weight). Consistent with this, proliferation and migration of NSCs did not show significant
differences in SLC39A8-p.393T knock-in mice compared with wild-types. In addition, SLC39A8-p.393T knock-in mice did not exhibit obvious behavioral and cognitive abnormalities, compared with wild-types. We thus focused on dendritic spine density, a featured characteristic that was frequently reported to be decreased in schizophrenia [28–30, 42]. Compared with wild-types, dendritic spine density of SLC39A8-p.393T knock-in mice was significantly decreased in cortex (\(P = 0.031\), one-tailed Student's t-test) (Fig. 5B). This finding is interesting as previous studies have repeatedly reported decreased dendritic spine density in schizophrenia [28–30, 42, 43]. Nonetheless, our finding is consistent with results observed in schizophrenia cases (i.e., decreased dendritic spine density) [29, 30, 42], indicating that SLC39A8-p.393T could regulate dendritic spine morphogenesis. This result also suggests that rs13107325 might confer schizophrenia risk by regulating zinc transport and dendritic spine density.

SLC39A8-p.393T knock-in mice did not show abnormal synaptic transmission
We investigated the effect of SLC39A8-p.393T on synaptic transmission by evaluating the properties of mEPSCs. We recorded mEPSCs from hippocampal CA1 pyramidal neurons by blocking sodium channels with 0.5 \(\mu\)M tetrodotoxin (TTX) on acute slices at postnatal days 18–21 mice. We found that neither the mEPSCs amplitude nor frequency of SLC39A8-p.393T knock-in mice showed any difference from that of wild-types (Fig. 6C–F), suggesting that the SLC39A8-p.393T has no effect on synaptic transmission regulation.

DISCUSSION
Among the schizophrenia risk variants identified by GWASs, the missense SNP rs13107325 is one of the most probable and promising
causal variants [5, 16]. Statistical fine-mapping [5], conservation analysis [21], functional annotation [22–24], physicochemical property differences of the amino acid encoded by different alleles of rs13107325, and functional characterizations supported the functionality and causality of this missense variant [25, 26]. Though lines of evidence support the potential pathogenicity of rs13107325 and several studies have explored the biological effect of this nonsynonymous variant in cell or animal models, whether rs13107325 is a pathogenic variant for schizophrenia remains unknown. To elucidate the role of rs13107325 in schizophrenia, we generated a knock-in mouse model by introducing a point mutation at SLC39A8-p.393T, which corresponds to human rs13107325 (SLC39A8-p.Ala393Thr). Considering the pleiotropic effect of rs13107325 [5, 17, 18, 24, 36], we assessed many rs13107325-associated phenotypes or traits using this knock-in mouse model, including body and brain weight, concentrations of metal ions (including cadmium, zinc, manganese, and iron) transported by SLC39A8, lipids, gene expression, behavioral and cognitive tests, proliferation and migration of NSCs, cortical development, dendritic spine density as well as synaptic transmission.

**Fig. 3** Subtle effect of SLC39A8-p.393T on cortical development. A, B Representative immunofluorescence images for four cortical layer-specific markers (TBR1, CTIP2, SOX2, SATB2) (P0 stage). C–F The subtle effect of SLC39A8-p.393T knock-in on cortical development. The statistical result of A was showed in C, D, the statistical result of B was showed in E, F. Two-tailed Student's test was used for statistical test. Data represent mean ± SD, n = 3 for (C–F).
Point mutation mouse model provides a pivotal opportunity to decipher the biological effects of missense risk variants in disease susceptibility and complex traits [44–47]. By evaluating the phenotypes and traits of SLC39A8-p.393T knock-in mice, we observed several interesting results. The first important finding of this study is that we found that SLC39A8-p.393T affected zinc concentration in brain and blood of the knock-in mice, validating the functionality of rs13107325. The second interesting finding is the subtle effect of SLC39A8-p.393T on cortical development, though the underlying mechanism remains unclear. The third intriguing finding is the decreased dendritic spine density observed in SLC39A8-p.393T knock-in mice. So far, the pathology of schizophrenia remains largely unknown. Nevertheless, accumulating evidence suggests that dysfunction of dendritic spines plays a crucial role in schizophrenia [28–30, 42, 43, 48, 49]. First, reduced dendritic spine density was frequently reported in schizophrenia [30, 42, 43]. Second, many schizophrenia risk genes have been reported to be involved in dendritic spine morphogenesis [50–53]. Third, a recent study by Radhakrishnan et al. demonstrated that decreased synaptic spine density is an intrinsic characteristic in schizophrenia [54]. These studies indicate that dysregulation of dendritic spines may have a critical role in schizophrenia. Consistent with these studies, we observed increased dendritic spine density in cortex in SLC39A8-p.393T knock-in compared with wild-type mice. It should be noted that the synaptic transmission of pyramidal neurons in hippocampus showed no differences in wild-type and SLC39A8-p.393T knock-in mice (Fig. 6C–F). More work is needed to investigate the role of rs13107325 in schizophrenia pathogenesis. In summary, we successfully linked the risk missense variant rs13107325 to a featured characteristic of schizophrenia by using the SLC39A8-p.393T knock-in mouse model. Our study clearly shows the importance of rs13107325 in dendritic spine morphogenesis, providing a reasonable biological explanation between rs13107325 and schizophrenia.

rs13107325 is a highly pleiotropic missense variant that showed strong association with many complex human traits and diseases, including schizophrenia [5, 15], blood lipids [36, 55],
SLC39A8 is widely expressed in diverse human tissues (data were from the Human Protein Atlas, https://www.proteinatlas.org/) (Supplementary Figure 10), with relatively high expression in lung, kidney, endometrium, placenta. This wide expression pattern suggests the important role of SLC39A8 in diverse human tissues. Consistent with this, multiple studies have demonstrated the pivotal physiological function of SLC39A8. For example, Besecker et al. revealed the important role of SLC39A8 in zinc-mediated cytoprotection in lung epithelia [63]. Studies by Zhang et al. and Fujishiro et al. showed that SLC39A8 has a crucial role in intracellular cadmium accumulation and cell toxicity [25, 64]. Liu et al. found that SLC39A8 regulates host defense by inhibiting NF-κB [65]. Ding et al. also found that SLC39A8 mediates the degradation of extracellular matrix via NF-κB signaling pathway [66]. Kim et al. demonstrated the critical role of SLC39A8 in osteoarthritis [67]. Of note, the study by Galvez-Peralta et al. indicated that SLC39A8 has an indispensable role for both organogenesis and hematopoiesis [68]. In addition to these findings, human genetic studies also showed the vital role of SLC39A8. Park et al. reported that SLC39A8 mutation was associated with congenital disorder of glycosylation by impairing β-1,4-galactosyltransferase and manganese uptake [69, 70]. Interestingly, a recent study by Tseng et al. showed that the human SLC39A8-p.391T missense variant (corresponds to the risk allele of rs13107325) reduced zinc transport (into the cell) in cell models [71], which was consistent with our observations in this study (i.e., zinc concentration was dysregulated in SLC39A8-p.391T knock-in mice compared with wild-types). Zn2+ has a critical role in the brain, including regulating synaptic plasticity through microtubule stability.

steatohepatitis [56], intelligence [57], Crohn’s disease [58], severe idiopathic scoliosis [59], brain structure [60–62], and others. The risk allele of rs13107325 only appears in European (8%) and American (5%) populations, but not in Asian and African populations (Supplementary Figure 2). Our previous study showed that the risk allele of rs13107325 might experience recent positive selection in Europeans, indicating this variant may act as a double-edged sword. The evolutionary selection shaped the frequency distribution of the risk allele of rs13107325 in world populations [21].

We did not detect differences in blood lipids, behaviors, and cognition between SLC39A8-p.393T knock-in and wild-type mice (Fig. 2). First, A possible explanation is the small number of the SLC39A8-p.393T knock-in and wild-type mice used in this study. Due to the huge time cost and expenditure, obtaining a large number of SLC39A8-p.393T knock-in mice is laborious and challenging. Thus, only limited SLC39A8-p.393T knock-in and wild-type mice were used for blood lipids detection, which may limit the statistical power of our study (considering the small effect of risk variants identified by GWASs). Second, the species differences (including genome, morphology, physiology, etc.) between human and mouse may also lead to the results observed in this study. Third, considering the polygenic nature of complex traits and diseases, it is likely that many variants (each has a small effect size) act synergistically to affect the complex traits. Nevertheless, we only considered a single variant in this study. Fourth, the advanced cognition such as associative memory, logical thinking, intelligence, is difficult to evaluate in mice. Fifth, we explored SLC39A8 expression in single cells from the human brain (CoDEx Viewer: http://solo.bmap.ucla.edu/shiny/webapp/, UCSC cell browser: https://cells.ucsc.edu/?ds=autism, and Transcriptomics Explorer of Allen brain map: https://celltypes.brain-map.org/rnaseq/human_ctx_smart-seq?selectedVisualization=Heatmap&colorByFeatureValue=Cell+Type&colorByFeatureValue=GAD1), and found that SLC39A8 is not widely expressed in different cell types of the human brain (Supplementary Figures 8, 9). Thus, more work is needed to further investigate how this missense variant confer risk of schizophrenia. Finally, the interaction between genetic and environmental factors also plays a pivotal role in regulating complex traits. Further work which considering both genetic and environmental factors are necessary to illuminate the role and mechanism of this missense variant in schizophrenia.

rs13107325 lies in the eighth exon of SLC39A8, which encodes ZIP8, a member of the solute-carrier-39 (SLC39) metal-transporter family. SLC39A8 is widely expressed in diverse human tissues (data were from the Human Protein Atlas, https://www.proteinatlas.org/) (Supplementary Figure 10–11), with relatively high expression in lung, kidney, endometrium, placenta. This wide expression pattern suggests the important role of SLC39A8 in diverse human tissues. Consistent with this, multiple studies have demonstrated the pivotal physiological function of SLC39A8. For example, Besecker et al. revealed the important role of SLC39A8 in zinc-mediated cytoprotection in lung epithelia [63]. Studies by Zhang et al. and Fujishiro et al. showed that SLC39A8 has a crucial role in intracellular cadmium accumulation and cell toxicity [25, 64]. Liu et al. found that SLC39A8 regulates host defense by inhibiting NF-κB [65]. Ding et al. also found that SLC39A8 mediates the degradation of extracellular matrix via NF-κB signaling pathway [66]. Kim et al. demonstrated the critical role of SLC39A8 in osteoarthritis [67]. Of note, the study by Galvez-Peralta et al. indicated that SLC39A8 has an indispensable role for both organogenesis and hematopoiesis [68]. In addition to these findings, human genetic studies also showed the vital role of SLC39A8. Park et al. reported that SLC39A8 mutation was associated with congenital disorder of glycosylation by impairing β-1,4-galactosyltransferase and manganese uptake [69, 70]. Interestingly, a recent study by Tseng et al. showed that the human SLC39A8-p.391T missense variant (corresponds to the risk allele of rs13107325) reduced zinc transport (into the cell) in cell models [71], which was consistent with our observations in this study (i.e., zinc concentration was dysregulated in SLC39A8-p.391T knock-in mice compared with wild-types). Zn2+ has a critical role in the brain, including regulating synaptic plasticity through microtubule stability.

![Fig. 5 SLC39A8-p.393T knock-in affected dendritic spine density. A Overview of dendritic spine analysis. B, C Dendritic spine density in cortex was significantly decreased in SLC39A8-p.393T knock-in mice compared with wild-types. One-tailed Student’s t-test was used for statistical test. Data represent mean ± SD, the number of neurons used for this quantitative analysis in WT and MT groups were n = 76 and n = 94 in cortex (B), n = 88 and n = 89 in hippocampus (C) (from 3 independent SLC39A8-p.393T knock-in mice and 3 SLC39A8-p.393A wild-type mice).](Image)
the action of Zn\textsuperscript{2+} synaptic transmission by reuptake of extracellular dopamine, and functional\cite{81}. The dopamine transporter (DAT) can terminate transmission in the prefrontal cortex of schizophrenia is hypothesized\cite{81}. We found that DAT levels might confer schizophrenia risk through affecting zinc transport.

Another amazing finding of this study is that only limited genes showed differential expression in cortical and hippocampal tissues of the SLC39A8-p.393T knock-in and wild-type mice. Only 12 and 4 DEGs were identified in cortical and hippocampal tissues, respectively, indicating the weak regulation effect of the SLC39A8-p.393T on gene expression in the brain. We also examined if the gene-editing process (i.e., SLC39A8-p.393T knock-in) affects Slc39a8 mRNA expression in mouse brain. We firstly checked if the gene-editing process affects SLC39A8 transcription using RNA sequencing data. We found that the gene-editing process did not affect Slc39a8 mRNA expression in the brain (Supplementary Figure 12A, B). Moreover, we also collected the cortical and hippocampal tissues from SLC39A8-p.393T knock-in mice and wild-types (5-month-old), and conducted quantitative PCR. Again, we found that Slc39a8 mRNA level did not show difference between SLC39A8-p.393T knock-in mice and wild-types (Supplementary Figure 12C, D). These findings suggest that rs13107325 may confer schizophrenia risk through affecting zinc transport.

In summary, we generated the SLC39A8-p.393T knock-in mouse model and we extensively characterized the phenotypes and traits of this knock-in mouse model. Although we did not detect significant differences in many traits (associated with rs13107325 in humans) in SLC39A8-p.393T knock-in and wild-type mice, we validated the functionality of rs13107325 and our study indicate that rs13107325 may confer schizophrenia risk by affecting dendritic spines.

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AUTHOR CONTRIBUTIONS

XJL conceived, designed, and supervised the whole study. SWL and CGM raised the animals, conducted genotyping, performed all experiments, and analyzed data under the supervision of XJL. CGM, SWL, and QXX collected the basic data (body weight, body length, brain weight, and iron concentration) of tested mouse. YFL, SWL, XYL, and XC performed dndritic spine density analysis. CGM, YXY, and XLD conducted transcriptome analysis and qPCR validation. SWL, XYL, and CW conducted behavioral tests. CGM and SWL conducted the proliferation and migration assays, RC and LPW conducted electrophysiological recording by acute slices of brain slices. YFY, WQL, LXL, CW, and NS provided critical comments on study design and manuscript writing. XJL and SWL interpreted the results and drafted the manuscript. XJL oversaw the project and finalized the manuscript. All authors revised the manuscript critically and approved the final version.

COMPETING INTERESTS

The authors declare no competing interests.

ADDITIONAL INFORMATION

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Correspondence and requests for materials should be addressed to Nengyi Sheng, Wenqi Li or Xiong-Jian Luo.

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