Schizophrenia Risk ZNF804A Interacts with its Associated Proteins to Modulate Dendritic Morphology and Synaptic Development

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Research

Keywords: schizophrenia, ZNF804A, ZNF804A binding proteins, dendritic morphology

DOI: https://doi.org/10.21203/rs.3.rs-113453/v1

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Abstract

Schizophrenia (SZ) is a devastating brain disease that affect about 1% of world population. Among the top genetic associations, zinc finger protein 804A (ZNF804A) gene encodes a zinc finger protein, associated with SZ and bipolar disorder (BD). Copy number variants (CNVs) of ZNF804A have been observed in patients with autism spectrum disorders (ASDs), anxiety disorder, and BD, suggesting that ZNF804A is a dosage sensitive gene for brain function. However, its molecular functions have not been fully determined. Our previous interactomic study revealed that ZNF804A interacts with multiple proteins to control protein translation and neural development. ZNF804A is localized in the cytoplasm and neurites in the human cortex and is expressed in various types of neurons, including pyramidal, dopaminergic, GABAergic, and Purkinje neurons in mouse brain. To further examine the effect of gene dosage of ZNF804A on neurite morphology, both knockdown and overexpression of ZNF804A in primary neuronal cells significantly attenuates dendritic morphology and synaptogenesis. To determine the effectors mediating these phenotypes, interestingly, three binding proteins of ZNF804A, galectin 1 (LGALS1), fasciculation and elongation protein zeta 1 (FEZ1) and ribosomal protein SA (RPSA), show different effects on reversing the deficits. LGALS1 and FEZ1 stimulate neurite outgrowth at basal level but RPSA shows no effect. Intriguingly, FEZ1 and RPSA but not LGALS1, can ameliorate ZNF804A-mediated dendritic abnormalities. Thus, our results uncover a critical post-mitotic role of ZNF804A in neurite and synaptic development relevant for neurodevelopmental pathologies.

Introduction

In 2008, O'Donovan and colleagues identified the ZNF804A as the first gene to reach the genome-wide significance associated with SZ [1]. The rs1344706 in the second intron of ZNF804A showed a strong association with both SZ and BD. The associations between ZNF804A and SZ were also reported by other groups [2–4]. In 2014, SZ Working Group of the Psychiatric Genomics Consortium compared 36,989 schizophrenia cases with 113,075 controls and identified 108 conservatively genomic loci that reached genome-wide significance[5]. ZNF804A is still among the one of top candidates. Besides single nucleotide polymorphisms (SNPs), CNVs of ZNF804A have been reported in patients with psychiatric diseases[3]. ZNF804A CNVs, including duplication and deletion, were also observed in patients with ASDs [6], supporting that ZNF804A risk is not only limited to SZ. These results also suggest that ZNF804A dosage is critical and altered expression may lead to various developmental disorders.

ZNF804A is highly expressed in brain tissues. The expression of ZNF804A in the brain increases during the early fetal stage and reaches the peak at mid-fatal stage (13–24 post-conceptual weeks), and decreases after birth [7]. We have also demonstrated that Zfp804a, a mouse homologous of human ZNF804, was highly expressed in the embryonic mouse brain and decreased expression in adulthood [8]. The human-induced pluripotent stem cells (iPSC) from SZ patients expressed ZNF804A at the early differentiation stage [9]. ZNF804A knockdown in cultured human neural progenitors (NPs) affects the expression of genes regulating cell adhesion, mitosis, neural differentiation, and inflammatory response [10]. ZNF804A regulates gene expression that relates to transforming growth factor β (TGFβ) signaling.
pathway [11]. The targets of ZNF804A include several SZ risk genes, including catechol-O-methyltransferase (COMT), phosphodiesterase 4B (PDE4B) and dopamine receptor D2 (DRD2) [12]. These results indicate that the gene-dosage of ZNF804A affects the transcriptome and cellular function.

Immunostaining of ZNF804A showed its expression along dendrites and in spines [13]. Downregulation of ZNF804A significantly reduced the neurite outgrowth and the density of dendritic spines in rat primary cortical neurons, suggesting an important role of ZNF804A on neurite outgrowth and maturation. We have demonstrated that Zfp804a, a mouse homologous of human ZNF804A, was highly expressed in the embryonic mouse brain and decreased expression in adulthood [8]. ZNF804A physically interacts with different proteins of SZ risk genes and developmental genes to regulate neurogenesis. However, how ZNF804A interacts with its binding partners to modulate neuritogenesis is unclear. This study tests how gene dosage of ZNF804A affects neurite outgrowth and synaptogenesis and shows that both upregulation and downregulation of ZNF804A could significantly impair dendritic morphology. Intriguingly, several ZNF804A binding proteins show distinct capacities on reversing the dendrite and synapse defects. Collectively, our study demonstrate that ZNF804A plays a critical role in the development of the nervous system and interacts with multiple genetic factors to contribute to cellular defects, which is relevant to the progression of psychiatric diseases.

Results

ZNF804A protein in mouse and human brain

In the human brain, the expression of ZNF804A mRNA increases from the embryonic to the early fetal stage and reaches a peak around the early-mid fetal stage. It decreases afterward and stays at a constant level until late adulthood [7]. As mRNA may not correlate with protein level, we first examined ZNF804A protein distribution in the brain. Consistent with our previous study, we observed the ZFP804A expression in primary cultured mouse neurons (Figure 1A). In the adult mouse brain, ZFP804A is expressed in neurons (Figure 1B) not in astrocytes (Figure 1C). Multiple types of neurons showed ZFP804A signal, including dopaminergic neurons (Figure 1D), GABAergic neurons (Figure 1E), and Purkinje neurons (Figure 1F). The immunostaining results confirmed that ZFP804A is localized in the nucleus, cytoplasm, and neurites of CA1 neurons (Figure 1G). Consistently, ZNF804A is localized in both nucleus and cytoplasm of neural cells in human brain sections (Figure 1H). Intriguingly, ZNF804A is highly enriched in some dendrites in human brain (Figure 1I), suggesting a potential role of ZNF804A in neurite development.

ZNF804A knockdown affects the neurite outgrowth and spine formation

ZNF804A protein is enriched in neurites (Figure 1A, 1G, 1I) and we have identified a group of ZNF804A interacting proteins involved in the neurite outgrowth using yeast-2-hybrid system (Y2H) [8] including...
FEZ1 and LGALS1. FEZ1 interacts with microtubules to enhance the extension of neurites [14, 15]. LGALS1 encodes galectin-1, which, when oxidized, loses its lectin activity that normally promotes neurite outgrowth and axonal regeneration [16, 17]. The neuronal migration deficits could be rescued by overexpression of the ZNF804A-interacting gene RPSA [8], indicating that protein–protein interactions could provide potential therapeutic targets for restoring deficits caused by genetic abnormalities. We hypothesize that these interactions can shape the neurite development. To test this hypothesis, we first confirmed the physical interaction of ZNF804A with 3 proteins, LGALS1, FEZ1 and RPSA, by co-immunoprecipitation (Figure 2A).

We next tested the effect of ZNF804A interacting proteins on Zfp804a-mediated neurite outgrowth. We co-transfected a FLAG tag vector or plasmids expressing LGALS1, FEZ1 and RPSA with a shRNA construct that we have shown the efficacy targeting Zfp804a [8] into primary mouse cortical neurons (Figure 2B-I) and analyzed the effects on dendritic morphology (Figure 3). Zfp804a shRNA significantly suppressed total number of neurite \((P=0.0239)\) (Figure 3A), total dendritic length \((P=0.0325)\) (Figure 3B), and the number of non-primary neurites \((P=0.0027)\) (Figure 3D), but not the number of primary neurite (Figure 3C). Sholl analysis confirmed the defect of neurite outgrowth caused by Zfp804a knockdown (Figure 3F, 3G, 3E).

Intriguingly, LGALS1, rather than FEZ1, rescued the neurite deficits caused by Zfp804a knockdown (Figure 3). LGALS1 recovered total number of neurite branch \((P=0.5843)\), dendritic length \((P=0.9999)\), and number of non-primary neurite \((P=0.408)\) (Figure 3A-D). The Sholl analysis indicated that LGALS1 increased the number of intersections between neurites and the consecutive circles in the Zfp804a downregulation neurons (Figure 3E, 3H-I). FEZ1 failed to rescue the neurite outgrowth deficits, and showed similar pattern as Zfp804a knockdown (Figure 3A-E, 3J-K). RPSA restored total number of neurites \((P=0.9981)\) and non-primary neurites \((P=0.7505)\), but not the dendritic length \((P=0.0001)\) compared to shControl+FLAG condition (Figure 3A-D). The Sholl analysis showed similar complexity of neurite outgrowth in RPSA overexpression group regardless Zpf804a levels (Figure 3E, 3L-M). Multiple comparisons of knockdown experiments were summarized in Supplemental table S1.

Dendritic spines are essential for receiving inputs from synapses. The density and plasticity of dendritic spines play a fundamental role to regulate neural functions. The morphological development of dendritic spines reflects their maturity [18]. A dendritic filopodia with a long neck and small head are considered to be an immature spine. It becomes shorter and reach to its maturity with a morphology of a mushroom head and a short neck [19]. The spine formation was significantly reduced by Zpf804a shRNA (Figure 4A). Downregulation of Zfp804a decreased the total spine density \((P=0.0003)\) and short spines \((P<0.0001)\) (Figure 4B-D), but not long spine number. LGALS1, FEZ1, or RPSA overexpression at basal level (with control shRNA) did not affect total dendritic spine density (Figure 4A-B). Interestingly, LGALS1, FEZ1, or RPSA ameliorated the defect of total spine density (Figure 4B), whereas LGALS1 and FEZ1 reversed the defect of short spine density comparing to the control group (Figure 4C). These data suggest that ZNF804A interacting proteins play different roles in ZNF804A-mediated dendritic/spine development.
ZNF804A overexpression reduces the neurite outgrowth and spine density

We previously demonstrated that overexpression of ZNF804A enhances translation rate [8] and duplication of ZNF804A is reported in patients with psychiatric illnesses [6]. To determine the biological function of a high level of ZNF804A in neurons, we co-transfected ZNF804A construct with plasmids expressing LGALS1, FEZ1, or RPSA into primary cortical neurons. Surprisingly, we observed a much stronger phenotype in neurite outgrowth in ZNF804A overexpressing neurons (Figure 5, 6).

Overexpression of ZNF804A significantly reduced the number of dendritic branches (P<0.0001) and total dendritic length of neurons (P<0.0001) (Figure 6A-B). The number of both primary and non-primary neurites were decreased (Figure 6C-D). The Sholl analysis support a significantly reduced number of neurite branches (Figure 6E-G).

LGALS1 promoted overall neurite outgrowth in the control group, significantly increasing the number of dendritic branches (Figure 6A) and dendritic length (Figure 6B). Specifically, LGALS1 overexpression significantly increased the number of non-primary neurites (Figure 6D), rather than primary neurites (Figure 6C). However, LGALS1 overexpression failed to restore aberrant neurite outgrowth in the ZNF804A overexpressing group (Figure 6A-D). The Sholl analysis confirms the insufficiency of LGALS1 in recovering ZNF804A overexpression-mediated dendritic defects (Figure 6E, 6H-I).

FEZ1 is known to directly bind to DISC1 protein, an important schizophrenia risk, to regulate neurite outgrowth [14]. Notably, we found that FEZ1 increased the dendritic length and the number of branches in ZNF804A overexpression group (Figure 6A-B). In vector-expressing cells, FEZ1 stimulates outgrowth in both primary and non-primary neurites similar as LGALS1 (Figure 6C-D). However, only FEZ1 overexpression reversed deficits of primary and non-primary neurites induced by ZNF804A overexpression (Figure 6C–D). Further Sholl analysis showed that FEZ1 overexpression restored the number of intersections between neurites and the proximal consecutive circles (Figure 6E, 6J-K)). This evidence indicated that FEZ1 prevented neurite outgrowth deficits caused by ZNF804A overexpression.

Interestingly, RPSA overexpression recovered the total number of neurites as well as both primary and non-primary neurites (Figure 6A, C-D). However, RPSA alone showed a trend to reduce the total dendritic length (P=0.695), and cannot rescue the deficits caused by ZNF804A overexpression (P=0.0007) (Figure 6B). The Sholl analysis confirmed our observation that the intersections between neurites and the consecutive circles did not reduce until 80 µm circle (Figure 6E, 6L-M).

We analyzed the spine density and morphology in the primary cultured mouse neurons with ZNF804A overexpression. The dendritic spines density showed significant reduction in the ZNF804A overexpressed mouse neurons (P=0.0328) (Figure 7A-B). Further analyzing spine morphology indicated that the short spines (P=0.0229) decreased significantly (Figure 7C) with an intact long/thin spine density (Figure 7D). Surprisingly, LGALS1, FEZ1, and RPSA could recovered the deficits of reduced spine density caused by
ZNF804A overexpression (Figure 7A-B). They showed similar number of short spines with a slightly elevated number of long/thin spine only in the PRSA overexpression group (Figure 7C-D). Multiple comparisons of overexpression experiments were summarized in Supplemental table S2.

Discussion

ZNF804A is highly expressed in NPs and reaches a peak at E14 in the embryonic mouse brain [8]. ZFP804A was expressed in neurons not in astrocytes (Fig. 1), suggesting ZNF804A plays an essential role in neurodevelopment. Here we demonstrated that in addition to modulating NP differentiation and neuronal migration, either upregulation or downregulation of ZNF804A significantly impaired dendritic and synaptic development. Intriguingly, upregulation produced a much severer neurite phenotype. Moreover, we showed that ZNF804A interacting proteins, LGALS1, FEZ1 and RPSA, exhibited different ability to alleviate the defects caused by abnormal ZNF804A levels. These data support that an optimal level of ZNF804A is required for normal dendritic morphology and suggest that ZNF804A interacts with different binding partners to modulate differentiation processes.

Studies have shown that neurite outgrowth deficits have been associated with dysfunctional schizophrenia risk genes, such as neuregulin 1 (NRG1), disrupted in schizophrenia 1 (DISC1), AKT serine/threonine kinase 1 (AKT1), and dystrobrevin binding protein 1 (DTNBP1) [14, 20, 21]. ZNF804A knockdown in cultured human neural progenitor cells affected the expression of genes related to cell adhesion [10]. Downregulation of Zfp804a repressed neuronal migration to the cortical plate in the mouse embryonic state [8]. Neurite outgrowth deficits were observed in the primary cultured mouse neuron with Zfp804a knockdown. The number of primary neurites, which originated from the soma, showed unaffected by reducing Zfp804a. The number of non-primary neurites significantly reduced as well as short total neurite length. Consistently, knockdown of ZNF804A attenuated neurite outgrowth in young human iPSC-derived neurons [13].

Psychiatric disorders often share associated risk loci with each other. A meta-analysis of eight mental disorders identified 109 risk SNP loci associated with at least two disorders. Most of autism spectrum disorder associated loci also involved SZ and BD [22]. Besides SNPs, CNVs, such as chromosomal deletion or duplication, have been implicated in psychiatric disorders. Both microdeletion and microduplication of 1q21.1 and 17p12 are associated with SZ and ASD [23]. CNV also happens within a single gene. Complex CNVs of contactin associated protein 2 (CNTNAP2) gene associated with several diseases across different phenotypes [24]. Interestingly, Steinberg and colleagues identified two ZNF804A deletions from a Scottish patient with schizophrenia and an Icelandic patient with anxiety, and one ZNF804A duplication in an Icelandic patient with BD [3]. ZNF804A overexpression significantly attenuated neurite outgrowth and dendritic spine formation. The reduction of the primary neurite generation is a distinct deficit between the overexpression and knockdown of ZNF804A. ZNF804A overexpression increases mRNA translation[8]. Our study reveals the complex mechanisms regulating neurite outgrowth by ZNF804A.
Interacting proteins can reveal the potential molecular and biological functions of a protein. More importantly, protein-protein interactions could serve as targets to rescue the disease phenotypes caused by risk genes. For example, the MDM2-p53 interaction became a valuable target for developing cancer therapy [25]. The interaction proteins of ZNF804A were explored with Y2H experiments in our previous study [8]. Two major functional groups, translation, and cell adhesion were clustered with multiple genes. LGALS1 and FEZ1 were reported with the capability of regulating neurite outgrowth [16, 17]. RPSA has a dual function as an RNA-binding protein and a laminin receptor to control cell adhesion [26]. Intriguingly, these interacting proteins exhibited distinct recovery potential against either overexpressed or downregulated ZNF804A. Both LGALS1 and FEZ1 stimulated overall neurite outgrowth. Only FEZ1 rescued the neurite outgrowth deficits caused by ZNF804A overexpression, whereas only LGALS1 recovered deficits induced by Zfp804a knockdown.

FEZ1 is known to physically interact with Disruptions in schizophrenia 1 (DISC1), a notable risk gene linked to SZ [14]. DISC1 plays a critical role in neurodevelopment, and its mutations lead to deficits in cell adhesion and neurite outgrowth [14, 27]. It regulates cell proliferation, differentiation, and migration through GSK3β/β-catenin pathway [28]. Dopamine D2 receptors affect neurites via dopamine D2 receptor-DISC1-GSK3β signaling [29]. Either hyper dopamine D2 receptor activation by over release of dopamine or blocked dopamine D2 receptors by its antagonist will lead to neurite outgrowth deficits [29]. ZNF804A physically interacts with FEZ1 to recover attenuated neurite outgrowth, indicating that FEZ1 is required for neurite elongation and fasciculation in mammals [30]. FEZ1 may function as a coordinator to facilitate neurite outgrowth, suggesting a potential interaction between ZNF804A and DISC1.

LGALS1 encodes protein galectin-1, a modulator of cell adhesion [31]. Galectin-1 is a therapeutic target of cancer treatment [32]. It also plays an important role in neuroprotection by regulating microglia activation in the central nervous system [33, 34]. Interestingly, Galectin-1 was found significantly higher in the unaffected siblings of SZ patients compared to both the patient group and the healthy control group [35]. Besides, Galectin-1 promoted neurite outgrowth and axonal regeneration [36, 37]. LGALS1, like FEZ1, stimulated nonprimary neurite outgrowth. But LGALS1 does not protect primary neurite deficit caused by ZNF804A overexpression. LGALS1 rescued the neurite deficits caused by Zfp804a downregulation, whereas FEZ1 did not. The opposite effect of LGALS1 implicated its relationship with ZNF804A and FEZ1. Overexpression of NLGN4X also recovered deficits of neurite length against the downregulation of ZNF804A in human neurons [13].

RPSA, encodes as a p40 ribosome-associated protein and a laminin receptor (37/67-kDa laminin receptor/ LAMR), involves in diverse biological functions. RPSA has been implicated in neurodegenerative diseases and developmental aberrations [38]. RPSA knockdown attenuated neurite outgrowth [39]. We previously demonstrated that RPSA rescued neuronal migration deficits caused by knockdown of Zfp804a in the mouse embryonic state [8]. Overexpression of RPSA also decreased the high level of cytoplasmic translation observed in neurons. Interestingly, RPSA overexpression recovered the neuron morphology by increasing the neurite number in both ZNF804A overexpression and Zfp804a knockdown group. However, it also inhibited the total dendritic length. Neurites with RPSA overexpression may take a
longer time to reach the same length as controls. Alternatively, lacking sufficient laminin may reduce the \textit{in vitro} neurite elongation. In summary, future therapeutic approaches of psychiatric diseases should be beneficial from careful studies of the critical gene-gene interactions.

\section*{Materials And Methods}

\subsection*{Animal and human postmortem samples}

All procedures on mice were reviewed and approved by the Pennsylvania State University institutional animal care and use committee (IACUC), under IACUC protocol number 44057-1. Wild type male and female C57BL/6N mice were obtained from Taconinc. Mice were housed by sex (2-5 mice per cage) in a room with a light/dark cycle at 12hr intervals, and provided \textit{ad libitum} access to food and water.

\subsection*{Neuronal culture and transfection}

Primary cortical neurons were cultured as described \cite{40}. Briefly, the embryonic cortex (embryonic day 14) was isolated in iced Hank's Balanced Salt Solution (HBSS) and digested with 0.25\% trypsin with DNase I for 5 min at 37\(^\circ\)C. After adding trypsin inhibitor, digested tissues were pipetted to break up into single cells. Cells were spun down at 1000 revolutions per minute (RPM) at 4\(^\circ\)C for 5 min. Spun cells were washed with HBSS and suspended with Dulbecco's Modified Eagle Medium (DMEM)/F-12 with 1\% Pen-Strep, 1\% L-glutamine, 1X N-2 and B27 supplements. Cells were plated into a 24-well plate with Poly-D-lysine pre-coated coverslips. Culture media were replaced with fresh media every other day.

Primary neurons were transient transfected with plasmids at day in vitro (DIV) 2 to overexpress \textit{ZNF804A}, or to knock down \textit{Zfp804a} with shRNA (5′-CAGAGAGAATTTGCTCGAAATG-3′). An empty vector for overexpression or a shRNA vector with scramble sequence (5′-GGCTCCCGTGAATTGGAATCC-3′) served as the negative control \cite{8}. \textit{ZNF804A} interacting partners, FEZ1, LGALS1, and RPSA, were co-transfected. A sham vector expressing FLAG tag were used as the negative control. The Calcium phosphate transfection was performed as described in Supplemental Information \cite{41}.

\subsection*{Immunostaining}

Cultured cells were grown on coverslips until mature. To fix cell samples, the culture medium was removed and washed with phosphate-buffered saline (PBS) for two times. Following the fixation with 4\% paraformaldehyde for 10 min at room temperature, cells were then washed with PBS twice. Cells were then blocked with 5\% donkey serum in PBS with 0.1\% Triton X-100 for 1 h. The primary antibodies were mixed in blocking buffer with appropriate dilution factors. After blocking, cells were incubated with primary antibodies overnight. Coverslips were washed with 0.3\% Triton X-100 in PBS for three times. Cells were then incubated with Alexa secondary antibodies that conjugated with fluorescein (Invitrogen). After
additional washing with PBS, cells were mounted with ProLong Gold antifade Reagents (Life Technologies).

**Sholl Analysis**

Immunostaining images were scanned by Carl Zeiss LSM 5 PASCAL confocal microscope. We analyzed the neurite number, length, and complexity of 40-60 individual neurons in each group by using the Sholl Analysis plugin integrated in Fiji [42]. The neurite derived from the soma of a neuron was considered as a primary neurite, and the neurite derived from a primary neurite was considered as a nonprimary neurite.

**Dendritic spine analysis**

To analyze the dendritic spine, a z-stack image was used to include all visible spines. Dendritic spines were classified as short (< 2µm) or long/thin (>2µm) based on their length. Spine density was calculated as the number spine per µm.

**Statistical Analysis**

Data were analyzed using Excel and SPSS software and are expressed as means ± standard error of the mean (SEM). Signicances between the experimental groups and control groups were analyzed by two-way ANOVA and multiple comparison test. The threshold of significance was set to *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$.

**List Of Abbreviations**

SZ, Schizophrenia

BD, biolar disorder

CNVs, copy number variants

ASDs, autism spectrum disorders

ZNF804A, zinc finger protein 804A

Zfp804a, zinc finger protein 804A

LGALS1, galectin 1

FEZ1, fasciculation and elongation protein zeta 1

RPSA, ribosomal protein SA
GABA, gamma-aminobutyric acid
iPSCs, induced pluripotent stem cells
SNP, single nucleotide polymorphism
NPs, neural progenitors
TGFβ, transforming growth factor β
COMT, catechol-O-methyltransferase
PDE4B, phosphodiesterase 4B
DRD2, dopamine receptor D2
DIV, day in vitro
shRNA, small hairpin RNA
NRG1, neuregulin 1
DISC1, disrupted in schizophrenia 1
AKT1, AKT serine/threonine kinase 1
DTNBP1, dystrobrevin binding protein 1
CNTNAP2, contactin associated protein 2
IACUC, institutional animal care and use committees
RPM, revolutions per minute
SEM, standard error of the mean
ANOVA, analysis of variance
HBSS, Hank's balanced salt solution
DMEM, Dulbecco's modified Eagle medium
PBS, phosphate-buffered saline
Y2H, yeast-2-hybrid system

Declarations
Ethics approval
The experiments using animals were approved by IACUC of Pennsylvania State University.

Consent for publication
Not applicable.

Availability of data and materials

Competing interests
The authors have declared no conflicts of interest in relation to the subject of this study.

Authors' contributions
Fengping Dong and Yingwei Mao designed the study; Fengping Dong, Joseph Mao, Miranda Chen and Joy Yoo performed the experiments and analyzed the data; Fengping Dong and Yingwei Mao wrote the manuscript.

Funding
This work was supported by the National Institute Of Mental Health of the National Institutes of Health under Award Number R21MH108983 and R01MH122556. The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH.

Acknowledgments
We thank Gong Chen, Bernhard Luscher, Timothy Jegla, Long Liu for technical support.

Availability of data and materials
The reagents are available from the corresponding author upon request.

References
1. O'Donovan MC, Craddock N, Norton N, Williams H, Peirce T, Moskvina V, Nikolov I, Hamshere M, Carroll L, Georgieva L, et al: Identification of loci associated with schizophrenia by genome-wide

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association and follow-up. Nat Genet 2008, 40:1053-1055.

2. Riley B, Thiselton D, Maher BS, Bigdeli T, Wormley B, McMichael GO, Fanous AH, Vladimirov V, O’Neill FA, Walsh D, Kendler KS: Replication of association between schizophrenia and ZNF804A in the Irish Case-Control Study of Schizophrenia sample. Mol Psychiatry 2010, 15:29-37.

3. Steinberg S, Mors O, Borglum AD, Gustafsson O, Werge T, Mortensen PB, Andreassen OA, Sigurdsson E, Thorgerisson TE, Bottcher Y, et al: Expanding the range of ZNF804A variants conferring risk of psychosis. Mol Psychiatry 2011, 16:59-66.

4. Williams HJ, Norton N, Dwyer S, Moskvina V, Nikolov I, Carroll L, Georgieva L, Williams NM, Morris DW, Quinn EM, et al: Fine mapping of ZNF804A and genome-wide significant evidence for its involvement in schizophrenia and bipolar disorder. Mol Psychiatry 2011, 16:429-441.

5. Schizophrenia Working Group of the Psychiatric Genomics C: Biological insights from 108 schizophrenia-associated genetic loci. Nature 2014, 511:421-427.

6. Anitha A, Thanseem I, Nakamura K, Vasu MM, Yamada K, Ueki T, Iwayama Y, Toyota T, Tsuchiya KJ, Iwata Y, et al: Zinc finger protein 804A (ZNF804A) and verbal deficits in individuals with autism. J Psychiatry Neurosci 2014, 39:130126.

7. Kang HJ, Kawasawa YI, Cheng F, Zhu Y, Xu X, Li M, Sousa AM, Pletikos M, Meyer KA, Sedmak G, et al: Spatio-temporal transcriptome of the human brain. Nature 2011, 478:483-489.

8. Zhou Y, Dong F, Lanz TA, Reinhart V, Li M, Liu L, Zou J, Xi HS, Mao Y: Interactome analysis reveals ZNF804A, a schizophrenia risk gene, as a novel component of protein translational machinery critical for embryonic neurodevelopment. Mol Psychiatry 2018, 23:952-962.

9. Pedrosa E, Sandler V, Shah A, Carroll R, Chang C, Rockowitz S, Guo X, Zheng D, Lachman HM: Development of patient-specific neurons in schizophrenia using induced pluripotent stem cells. J Neurogenet 2011, 25:88-103.

10. Hill MJ, Jeffries AR, Dobson RJ, Price J, Bray NJ: Knockdown of the psychosis susceptibility gene ZNF804A alters expression of genes involved in cell adhesion. Hum Mol Genet 2012, 21:1018-1024.

11. Umeda-Yano S, Hashimoto R, Yamamori H, Okada T, Yasuda Y, Ohi K, Fukumoto M, Ito A, Takeda M: The regulation of gene expression involved in TGF-beta signaling by ZNF804A, a risk gene for schizophrenia. Schizophr Res 2013.

12. Girgenti MJ, LoTurco JJ, Maher BJ: ZNF804a regulates expression of the schizophrenia-associated genes PRSS16, COMT, PDE4B, and DRD2. PLoS One 2012, 7:e32404.

13. Deans PJM, Raval P, Sellers KJ, Gatford NJF, Halai S, Duarte RRR, Shum C, Warre-Cornish K, Kaplun VE, Cocks G, et al: Psychosis Risk Candidate ZNF804A Localizes to Synapses and Regulates Neurite Formation and Dendritic Spine Structure. Biol Psychiatry 2017, 82:49-61.

14. Miyoshi K, Honda A, Baba K, Taniguchi M, Oono K, Fujita T, Kuroda S, Katayama T, Tohyama M: Disrupted-In-Schizophrenia 1, a candidate gene for schizophrenia, participates in neurite outgrowth. Mol Psychiatry 2003, 8:685-694.

15. Suzuki T, Okada Y, Semba S, Orba Y, Yamanouchi S, Endo S, Tanaka S, Fujita T, Kuroda S, Nagashima K, Sawa H: Identification of FEZ1 as a protein that interacts with JC virus agnoprotein and
microtubules: role of agnoprotein-induced dissociation of FEZ1 from microtubules in viral propagation. *J Biol Chem* 2005, **280**:24948-24956.

16. Puttagunta R, Di Giovanni S: **Retinoic acid signaling in axonal regeneration.** *Frontiers in Molecular Neuroscience* 2012, **4**.

17. Camby I, Le Mercier M, Lefranc F, Kiss R: **Galectin-1: a small protein with major functions.** *Glycobiology* 2006, **16**:137R-157R.

18. Nimchinsky EA, Sabatini BL, Svoboda K: **Structure and function of dendritic spines.** *Annu Rev Physiol* 2002, **64**:313-353.

19. Qiao H, Li MX, Xu C, Chen HB, An SC, Ma XM: **Dendritic Spines in Depression: What We Learned from Animal Models.** *Neural Plast* 2016, **2016**:8056370.

20. Bellon A: **New genes associated with schizophrenia in neurite formation: a review of cell culture experiments.** *Mol Psychiatry* 2007, **12**:620-629.

21. Ma X, Fei E, Fu C, Ren H, Wang G: **Dysbindin-1, a schizophrenia-related protein, facilitates neurite outgrowth by promoting the transcriptional activity of p53.** *Mol Psychiatry* 2011, **16**:1105-1116.

22. Lee PH, Anttila V, Won H, Feng Y-CA, Rosenthal J, Zhu Z, Tucker-Drob EM, Nivard MG, Grotzinger AD, Posthuma D, et al: **Genomic Relationships, Novel Loci, and Pleiotropic Mechanisms across Eight Psychiatric Disorders.** *Cell* 2019, **179**:1469-1482.e1411.

23. Rees E, Walters JT, Georgieva L, Isles AR, Chambert KD, Richards AL, Mahoney-Davies G, Legge SE, Moran JL, McCarroll SA, et al: **Analysis of copy number variations at 15 schizophrenia-associated loci.** *Br J Psychiatry* 2014, **204**:108-114.

24. Toma C, Pierce KD, Shaw AD, Heath A, Mitchell PB, Schofield PR, Fullerton JM: **Comprehensive cross-disorder analyses of CNTNAP2 suggest it is unlikely to be a primary risk gene for psychiatric disorders.** *PLoS Genet* 2018, **14**:e1007535.

25. Trino S, De Luca L, Laurennzana I, Caivano A, Del Vecchio L, Martinelli G, Musto P: **P53-MDM2 Pathway: Evidences for A New Targeted Therapeutic Approach in B-Acute Lymphoblastic Leukemia.** *Front Pharmacol* 2016, **7**:491.

26. Kleinman HK, Ogle RC, Cannon FB, Little CD, Sweeney TM, Luckenbill-Edds L: **Laminin receptors for neurite formation.** *Proc Natl Acad Sci U S A* 1988, **85**:1282-1286.

27. Hattori T, Shimizu S, Koyama Y, Yamada K, Kuwahara R, Kumamoto N, Matsuzaki S, Ito A, Katayama T, Tohyama M: **DISC1 regulates cell-cell adhesion, cell-matrix adhesion and neurite outgrowth.** *Mol Psychiatry* 2010, **15**:778, 798-809.

28. Mao Y, Ge X, Frank CL, Madison JM, Koehler AN, Doud MK, Tassa C, Berry EM, Soda T, Singh KK, et al: **Disrupted in schizophrenia 1 regulates neuronal progenitor proliferation via modulation of GSK3beta/beta-catenin signaling.** *Cell* 2009, **136**:1017-1031.

29. Huang XF, Song X: **Effects of antipsychotic drugs on neurites relevant to schizophrenia treatment.** *Med Res Rev* 2019, **39**:386-403.
30. Okumura F, Hatakeyama S, Matsumoto M, Kamura T, Nakayama KI: **Functional regulation of FEZ1 by the U-box-type ubiquitin ligase E4B contributes to neuritogenesis.** *J Biol Chem* 2004, **279**:53533-53543.

31. Hughes RC: **Galectins as modulators of cell adhesion.** *Biochimie* 2001, **83**:667-676.

32. Su YL, Luo HL, Huang CC, Liu TT, Huang EY, Sung MT, Lin JJ, Chiang PH, Chen YT, Kang CH, Cheng YT: **Galectin-1 Overexpression Activates the FAK/PI3K/AKT/mTOR Pathway and Is Correlated with Upper Urinary Urothelial Carcinoma Progression and Survival.** *Cells* 2020, **9**.

33. Nonaka M, Fukuda M: **Galectin-1 for neuroprotection?** *Immuinity* 2012, **37**:187-189.

34. Aalinkeel R, Mahajan SD: **Neuroprotective role of galectin-1 in central nervous system pathophysiology.** *Neural Regen Res* 2016, **11**:896-897.

35. Yuksel RN, Goverti D, Kahve AC, Cakmak IB, Yuksel C, Goka E: **Galectin-1 and Galectin-3 Levels in Patients with Schizophrenia and their Unaffected Siblings.** *Psychiatr Q* 2020.

36. Puche AC, Poirier F, Hair M, Bartlett PF, Key B: **Role of galectin-1 in the developing mouse olfactory system.** *Dev Biol* 1996, **179**:274-287.

37. Horie H, Inagaki Y, Sohma Y, Nozawa R, Okawa K, Hasegawa M, Muramatsu N, Kawano H, Horie M, Koyama H, et al: **Galectin-1 regulates initial axonal growth in peripheral nerves after axotomy.** *J Neurosci* 1999, **19**:9964-9974.

38. DiGiacomo V, Meruelo D: **Looking into laminin receptor: critical discussion regarding the non-integrin 37/67-kDa laminin receptor/RPSA protein.** *Biol Rev Camb Philos Soc* 2016, **91**:288-310.

39. Blazejewski SM, Bennison SA, Ha NT, Liu X, Smith TH, Dougherty KJ, Toyo-oka K: **PEDF-Rpsa-Ltga6 signaling regulates cortical neuronal morphogenesis.** *bioRxiv* 2020:2020.2001.2006.895672.

40. Sanada K, Tsai LH: **G protein betagamma subunits and AGS3 control spindle orientation and asymmetric cell fate of cerebral cortical progenitors.** *Cell* 2005, **122**:119-131.

41. Jiang M, Chen G: **High Ca2+-phosphate transfection efficiency in low-density neuronal cultures.** *Nat Protoc* 2006, **1**:695-700.

42. Schindelin J, Arganda-Carreras I, Frise E, Kaynig V, Longair M, Pietzsch T, Preibisch S, Rueden C, Saalfeld S, Schmid B, et al: **Fiji: an open-source platform for biological-image analysis.** *Nat Methods* 2012, **9**:676-682.

**Figures**

Figure 1

ZN804A antibody immunostaining demonstrated the localization of ZFP804A in primary cultured mouse neurons (A). ZFP804A is highly expressed in the nuclear as well as in cytoplasm and neurites. In the mouse brain, ZFP804A is exclusively expressed in neurons (B) with no detectable signal in astrocytes
(C) ZFP804A expressed in different types of neurons, for example dopaminergic neurons (D), GABAergic neurons (E), and Purkinje cells (F). ZFP804A is highly expressed in neurites (arrows) (G). Bar = 10 µm. (H-I) Immunostaining demonstrates that ZNF804A is highly expressed in nucleus of human neurons (H) and neurites (I). Bar = 10 µm.

**Figure 2**

(A) ZNF804A protein physically interacts with LGALS1, FEZ1, and RPSA. (B-I) Zfp804a shRNA or a scramble shRNA are co-transfected with constructs expressing tag (B-C), LGALS1 (D-E), FEZ1 (F-G) or RPSA (H-I) separately into primary mouse cortical neurons. The transfected neurons are stained with GFP (green). Bar = 50 µm.
Figure 3

Zfp804a knockdown in differentiating neurons decreases number of branches (A) and total dendritic length (B). LGALS1 restores both neurite number and neurite length deficits caused by downregulation of Zfp804a. Zfp804a knockdown does not affect primary neurite number (C). However, it significantly reduces the nonprimary neurite number (D). LGALS1 and RPSA rescue the deficits of nonprimary number reduction. The Sholl analysis indicates the complexity of the neurite outgrowth (E), and illustrates representative co-transfected neurons with concentric circles (F-M). The interval between adjacent consecutive circles is 10 μm (n = 70).
Figure 4

The spine density of transient co-transfect mouse neurons was measured (A) Zfp804a knockdown significantly reduces spine formation (B-D), LGALS1, FEZ1, and RPSA reverse the reduction of total spine number caused by Zfp804a downregulation (B). LGALS1 and FEZ1 restore short spines as well as long/thin spines (C-D). Bar = 2 µm.
Figure 5

ZNF804A and empty GFP overexpression vectors were co-transfected with vectors expressing tags (A-B), LGALS1 (C-D), FEZ1 (E-F) or RPSA (G-H) separately into primary neurons. The transfected neurons were stained with GFP (green). Bar = 50 µm.
Figure 6

ZNF804A and empty GFP overexpression vectors were co-transfected with vectors expressing tags (A-B), LGALS1 (C-D), FEZ1 (E-F) or RPSA (G-H) separately into primary neurons. The transfected neurons were stained with GFP (green). Bar = 50 µm.
The spine density of transient co-transfect mouse neurons was measured (A). ZNF804A overexpression significantly reduces total spine formation (B) and the number of short spines (C), but not long/thin spines (D). LGALS1, FEZ1, and RPSA alleviate the deficits of spine formation caused by ZNF804A overexpression (B-C). Bar = 2 µm.

**Supplementary Files**

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