Pathological oligodendrocyte precursor cells revealed in human schizophrenic brains and trigger schizophrenia-like behaviors and synaptic defects in genetic animal model

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Although the link of white matter to pathophysiology of schizophrenia is documented, loss of myelin is not detected in patients at the early stages of the disease, suggesting that pathological evolution of schizophrenia may occur before significant myelin loss. Disrupted-in-schizophrenia-1 (DISC1) protein is highly expressed in oligodendrocyte precursor cells (OPCs) and regulates their maturation. Recently, DISC1-Δ3, a major DISC1 variant that lacks exon 3, has been identified in schizophrenia patients, although its pathological significance remains unknown. In this study, we detected in schizophrenia patients a previously unidentified pathological phenotype of OPCs exhibiting excessive branching. We replicated this phenotype by generating a mouse strain expressing DISC1-Δ3 gene in OPCs. We further demonstrated that pathological OPCs, rather than myelin defects, drive the onset of schizophrenic phenotype by hyperactivating OPCs’ Wnt/β-catenin pathway, which consequently upregulates Wnt Inhibitory Factor 1 (Wif1), leading to the aberrant synaptic formation and neuronal activity. Suppressing Wif1 in OPCs rescues synaptic loss and behavioral disorders in DISC1-Δ3 mice. Our findings reveal the pathogenic role of OPC-specific DISC1-Δ3 variant in the onset of schizophrenia and highlight the therapeutic potential of Wif1 as an alternative target for the treatment of this disease.

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INTRODUCTION

Schizophrenia, a complex and severe psychiatric condition [1], is one of the 15 leading causes of disability, affecting 1 in 300 people worldwide [2, 3]. Although highly heritable, the pathophysiological mechanisms of schizophrenia have not been well understood. Clinical evidence has linked white matter abnormalities to schizophrenia symptoms [4]. Pathophysiological changes include impaired oligodendrocyte differentiation, myelination, and white matter loss [5–7]. However, several diffusion tensor imaging (DTI) studies found no changes in the white matter volume during the early stages of schizophrenia [8–11], suggesting that early pathogenesis of schizophrenia may precede significant myelin loss.

DISC1 emerged as a schizophrenia susceptible gene since its genomic disruption, while frameshift mutation, or single nucleotide polymorphisms (SNPs) were associated with familial schizophrenia [12–14]. Further studies revealed that DISC1 acts as a scaffold protein regulating multiple schizophrenia-related neuronal development processes through its protein partners [15–18]. However, recent advances showed that DISC1 dysfunction also affects OPC maturation and subsequent myelination [19, 20], while RNA-sequencing analyses in both humans and mice demonstrated that OPCs express higher levels of DISC1 when compared to neurons or mature oligodendrocytes (OLs) [21, 22], raising a possibility that the high presence of DISC1 in the OPCs may contribute to the pathogenesis of schizophrenia.

In addition, previous studies showed that DISC1 undergoes extensive alternative splicing, which is highly regulated by schizophrenia-associated SNPs [23, 24]. Several DISC1 splicing variants are upregulated in the hippocampi of schizophrenia patients [24–26], where significant pathological changes occur at the clinical high risk (CHR) stage of the onset of schizophrenia [27]. Among these variants, DISC1-Δ3, a splicing variant that lacks exon 3, is one of the most upregulated DISC transcripts in multiple brain regions [24, 26], especially in individuals with intronic SNP rs821597 [24]. DISC1-Δ3 transcript translates into a short DISC1 protein isoform lacking the binding sites with several protein partners [26, 28], yet little is known of its role in oligodendroglia or in the pathogenesis of schizophrenia.

In the present study, we revealed previously unknown pathological hypertrophic OPCs in schizophrenia patients. We were able to replicate these pathological OPCs in a newly
generated transgenic mouse strain, which mimics the enhanced DISC1 exon 3 splicing in patients by removing the DISC1 exon 3 from a single allele in oligodendroglial lineage cells. Mechanistically, we demonstrated that mice with enhanced DISC1-Δ3 variant expression in OPCs display schizophrenia-like behaviors and synaptic defects, both driven by the overactivated Wnt/β-catenin-Wnt inhibitory factor 1 (Wif1) cascade. Our results provide an alternative insight into the critical role of dysfunctional OPCs in pathogenesis of schizophrenia and highlight a molecular target, Wif1, for developing potential therapeutic strategies.

RESULTS
OPCs are hypertrophic in schizophrenia patients
We examined the histological properties of the OPCs in postmortem brain tissues of schizophrenia patients and age-matched healthy controls (Supplementary Fig. 1a). The Sholl analysis of NG2-positive OPCs revealed previously unidentified hypertrophic morphotype in the hippocampus, prefrontal cortex, and amygdala of schizophrenia patients when compared to the age-matched healthy controls in both human paraffin tissue sections (Fig. 1a-i) and frozen sections (Supplementary Fig. 1b–g). The OPCs in post-mortem tissues of schizophrenia patients displayed a significant increase in the mean number of branches (increased by 73.4 ± 6.9%, 35.3 ± 4.0%, and 21.8 ± 3.9% respectively in the hippocampus, prefrontal cortex, and amygdala in paraffin human brain tissue sections, Fig. 1a–i; increased by 42.1 ± 12.5% and 40.6 ± 14.3%, respectively in the hippocampus and prefrontal cortex in frozen sections, Supplementary Fig. 1b–g) and greater mean branch length (increased by 64.7 ± 7.6%, 31.3 ± 3.1% and 24.9 ± 5.3% in paraffin tissue sections, Fig. 1a–i; increased by 42.9 ± 9.7% and 57.8 ± 13.8% in frozen sections, Supplementary Fig. 1b–g). In contrast, neither the number of NG2-positive OPCs (paraffin section, Fig. 1b, e, h; frozen section, Supplementary Fig. 1c, f) nor the number of OLG2-positive oligodendroglial cells (paraffin section, Fig. 1j, k) per area unit was affected in schizophrenia patients. This is the first time demonstration of hypertrophic pathological OPCs in schizophrenia patients.

Enhanced DISC1-Δ3 expression in the oligodendroglia replicates the hypertrophic OPC in patients
DISC1 is a schizophrenia risk gene and regulates oligodendroglial development [19, 20]. The gene sequence of DISC1 is highly conserved between humans and mice (Supplementary Fig. 2a). Re-analyzing RNA-sequencing libraries [21, 22] showed that DISC1 expression in human and mouse OPCs is much higher than in positive OPCs (paraf...
period in this study) may exert prolonged effects on synaptic development in later life (e.g., puberty in this study), we induced the DISC1-Δ3 deletion at a later stage (between P40 - P45), when the majority of myelin sheaths are established. We assessed the histological and behavioral changes at P50, when no OPC differentiation or myelin deficiency was found (Fig. 3a–f, Supplementary Fig. 3a–c). In all three experiments, the NG2+ areas per cell in DISC1-Δ3 mice at the endpoints increased by 110.1 ± 20.5%, 359.7 ± 46.7%, and 250.2 ± 51.7% respectively (Fig. 3g, h, Supplementary Fig. 3d, e), with a more hypertrophic morphology as shown by Sholl analysis (Fig. 3i). These OPCs were in close contact with the cell bodies of NeuN+ neurons with synaptic defects (Fig. 3j–n). However, the number of Olig2+ cells remained unchanged in all three experiments (Supplementary Fig. 3f).

The absence of myelin deficiency allowed us to investigate the contribution of DISC1-Δ3 OPCs to the above mentioned synaptic defects and schizophrenia-like behaviors. We found that pre- and post-synaptic compartments were significantly reduced in the hippocampus in all three experimental groups (Fig. 4a, Supplementary Fig. 4). Golgi staining confirmed decreased number of dendritic spines in DISC1-Δ3 OPCs in the amygdala. n = 30 cells from samples per group. j Immunostaining of Olig2 in the hippocampus. Scale bar, 50 µm. k Quantification of Olig2 in the hippocampus, cortex, and amygdala. n = 5 samples per group. Plots show individual data and mean ± SD, or mean ± SEM in Sholl analysis results. n.s., not significant, *p < 0.05, **p < 0.01, ****p < 0.0001; paired t-test, or two-way ANOVA for Sholl analysis.

In order to further confirm that aberrant OPCs but not mature OLs contribute to the neuronal defects during the onset of schizophrenia, we cross-bred the DISC1exon3flx mice with the OL-specific PLPCreERT mice to obtain PLPCreERT:DISC1exon3flx/+ mice (OL

Fig. 1 Hypertrophic OPCs in schizophrenia patients. a Immunohistochemistry of NG2 in the paraffin-embedded hippocampal sections of healthy controls and schizophrenia patients. Arrowheads highlight NG2+ OPCs. Lower panel, example of Sholl analysis. b Quantification of NG2+ OPC number in the hippocampus. n = 5 samples per group. c Sholl analysis of NG2+ OPC in the hippocampus. n = 30 cells from samples per group. d Immunohistochemistry of NG2 in the paraffin-embedded cortical sections of healthy controls and schizophrenia patients. Arrowheads highlight NG2+ OPCs. Lower panel, example of Sholl analysis. e Quantification of NG2+ OPC number in the cortex. n = 5 samples per group. f Sholl analysis of NG2+ OPC in the cortex. n = 30 cells from samples per group. g Immunohistochemistry of NG2 in the paraffin-embedded amygdala sections of healthy controls and schizophrenia patients. Arrowheads highlight NG2+ OPCs. Scale bar, 50 µm. Lower panel, example of Sholl analysis. h Quantification of NG2+ OPC number in the amygdala. n = 5 samples per group. i Sholl analysis of NG2+ OPC in the amygdala. n = 30 cells from samples per group. j Immunostaining of Olig2 in the hippocampus. Scale bar, 50 µm. k Quantification of Olig2 in the hippocampus, cortex, and amygdala. n = 5 samples per group. Plots show individual data and mean ± SD, or mean ± SEM in Sholl analysis results. n.s., not significant, *p < 0.05, **p < 0.01, ****p < 0.0001; paired t-test, or two-way ANOVA for Sholl analysis.
DISC1-Δ3, Fig. 4g). Tamoxifen administration specifically removed a single copy of the DISC1 exon 3 from one allele in OLs (DISC1-Δ3 OLs, Fig. 4g). In the hippocampus of these mice at P21, enhanced expression of DISC1-Δ3 in OLs did not change the numbers of SYN1+ synaptic puncta (Fig. 4h). In addition, neither the morphology of PDGFRα+ OPCs, MBP+ myelin structures, nor numbers of CC1+ mature OLs and Olig2+ oligodendroglial lineage cells (Fig. 4i, j; Supplementary Fig. 3g, h) were affected. These results suggest an OPC-specific manner to initiate schizophrenia-like pathological changes in the DISC1-Δ3 mice.
Subsequently, we performed in vitro experiments to confirm the direct impact of DISC1-Δ3 OPCs on neurons. Primary OPCs were isolated from the brains of DISC1-Δ3 and non-CreERT wild-type mice at P7 by immunopanning. They were either co-cultured with hippocampal neurons, or used for medium conditioning to treat the hippocampal neurons. Cultured DISC1-Δ3 OPCs showed hypertrophic morphology similar to that observed in DISC1-Δ3 mice (Fig. 4k). Neuronal synapse formation in cells co-cultured with DISC1-Δ3 OPC or exposed to DISC1-Δ3 OPC generated conditioned medium was significantly suppressed (the number of synapses decreased by 58.0 ± 13.3% and 23.5 ± 5.2% respectively, Fig. 4l, m).

Collectively, our results demonstrate that pathological OPCs and not myelin defects are the main contributors to the onset of schizophrenia-like symptoms. In addition, DISC1-Δ3 OPCs affect neuronal synaptic formation in a paracrine manner.

**Hyperactivity of Wnt/β-catenin pathway in OPCs from schizophrenia patients and DISC1-Δ3 mice**

To gain an insight into underlying molecular mechanisms, RNA-sequencing was performed in the OPCs from DISC1-Δ3 and non-CreERT wildtype mice (Supplementary Fig. 5a-c). The Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis indicated significantly increased activity of Wnt/β-catenin pathway in DISC1-Δ3 OPCs (Supplementary Fig. 5c). This was deduced from a 56.9 ± 14.0% reduction in phosphorylated β-catenin (the degraded form of Wnt signaling stimulator), 44.8 ± 15.0% reduction in autophosphorylation of glycogen synthase kinase 3 β (GSK3β) at Tyrosine 216 (Y216), the functional form of an inhibitor of the Wnt/β-catenin pathway, and a 76.3 ± 20.3% increase in the phosphorylated GSK3β at Serine 9 (S9, Fig. 5a) that inactivates GSK3β [33–35]. In addition, a significant upregulation of Wnt downstream genes in multiple cellular processes [40], we postulate that the DISC1-Δ3 in OPCs mediates a ‘gain-of-function’ to promote the hyperactivity of the Wnt/β-catenin signaling pathway in schizophrenia brains.

However, the morphological changes in OPCs cannot be achieved either in vivo or in vitro by OPC-specific conditional deletion of the obligate Wnt pathway inhibitor, adenosomatous polyposis coli (APC) (Supplementary Fig. 5e, f). This suggests that Wnt/β-catenin pathway is probably not involved in generation of the DISC1-Δ3 OPCs morphological phenotype.

**Wnt-driven Wif1 overexpression impairs synaptic formation**

As Wnt/β-catenin pathway activity governs the expression of downstream genes in multiple cellular processes [40], we investigated how hyperactive Wnt signaling in DISC1-Δ3 OPCs affects synaptic formation. Differential expression analysis showed that DISC1-Δ3 altered the expression of several secretory protein-coding genes (Supplementary Fig. 5g, h), especially Wif1 (420.1 ± 21.4-fold increase, Supplementary Fig. 5i), which is a downstream target of Wnt and an inhibitor of the Wnt pathway [41]. Recently, it has been proposed that upregulated Wif1 forms part of a negative feedback mechanism to counteract the excessive activities of the Wnt signaling pathway in OPCs [42].

Increased expression of Wif1 was detected by immunostaining, ELISA, western blot, and qPCR (55.2 ± 21.4%, 23.3 ± 3.9%, 100.2 ± 38.9%, and 500.0 ± 163.1% increase, respectively, Fig. 5f-i). Consistent with our findings in DISC1-Δ3 mice, WIFI1 intensity was significantly increased by 111.4 ± 34.5% in the hippocampus of schizophrenia patients when compared to that in the age-matched healthy controls (Fig. 5j), which is in agreement with previous observations that WIFI1 is altered in schizophrenia postmortem brains [43, 44].

Since Wif1 is a secreted factor, the overproduction of Wif1 in OPCs may affect other Wnt-dependent processes in adjacent neurons, such as synaptogenesis [45]. To address this question, we measured the activation of Wnt signaling pathway in acutely isolated neurons from P10 DISC1-Δ3 and non-CreERT wildtype OPCs.
mice. We found that the target genes of the non-canonical Wnt pathways [46] were downregulated in the neurons from DISC1-Δ3 mice, whereas the canonical Wnt/β-catenin pathway remains unchanged (Fig. 5k). Next, we treated primary wild-type hippocampal neurons with the synaptogenic Wnt ligand Wnt7a [47] in the presence of Wif1 protein. Wif1 treatment resulted in an 80.8 ± 10.6% reduction in the number of SYN1+ synaptic puncta compared to Wnt7a-treatment alone (Fig. 5l). This was accompanied by a decrease in Wnt/Ca^{2+} signaling elements: 55.9 ± 5.0% reduction in CaMK II phosphorylation and suppressed mRNA expression of non-canonical Wnt target genes (Supplementary Fig. 5j, k). Similar changes occur in neurons from DISC1-Δ3 mice, which agrees with previous studies that the canonical and non-canonical Wnt pathways regulate synaptogenesis [48–50]. Therefore, our data suggest that the overproduction of Wif1 protein from DISC1-Δ3 results in the inhibition of synaptogenesis mediated by non-canonical Wnt pathway.
Downregulation of Wif1 rescues synaptic defects and behavioral abnormalities in DISC1-Δ3 mice

To further verify whether manipulating Wif1 expression can rescue synaptic defects and behavioral abnormalities in DISC1-Δ3 mice, we knocked down Wif1 in the DISC1-Δ3 OPCs by microinjecting Wif1 shRNA retrovirus into the hippocampus [51]. The infection efficiency was confirmed by the co-expression of retrovirus-driven GFP and the NG2creERT-driven tdTomato (Supplementary Fig. 6a). The infection specificity for oligodendroglial cells was validated by co-labeling of GFP+ with NG2creERT:tdTomato+ (82.13 ± 1.45%)
until the onset of schizophrenia-related pathological changes, which may explain the results of previous imaging studies. Schizophrenia is usually diagnosed between late adolescence and early thirties [53, 54], when myelination is still ongoing [55, 56]. Any abnormal activation of Wnt/β-catenin pathway in OPCs within this age may affect their differentiation and hinder myelination [57, 58]. Indeed, our findings in schizophrenia patients and mouse model show that Wnt-signaling elements in the OPCs are hyperactive especially at the initiation of schizophrenia-like pathology in mice, suggesting that defects in myelination may follow later when disease evolves [9, 10]. Although we still lack direct evidence linking OPC hypertrophies to Wnt-signaling hyperactivation, as well as their association with DISC1-Δ3 splicing in schizophrenia patients, these early changes in OPCs, (found in both schizophrenia patients and mouse model), may either initiate or contribute to the onset of schizophrenia.

Our study further corroborates the multi-functional role of OPCs in the central nervous system (CNS). In other neurological disorders, such as multiple sclerosis, hypoxic-ischemic encephalopathy, and psychiatric diseases, OPCs not only contribute to aberrant myelin formation [39, 57], but are also involved in the regulation of blood-brain barrier integrity [42], CNS immune regulation [59, 60] and the behavioral outcomes [61, 62]. In the present study, we provided new evidence that selective enhancement of DISC1 exon 3 splicing in OPCs, disrupts the formation of vGLUT1+ excitatory synapse but not vGAT+ inhibitory synapse. This leads to excitatory/inhibitory synaptic imbalance (E/I imbalance), which significantly contributes to the pathophysiology of schizophrenia [63]. The E/I imbalance is well documented in the hippocampus of schizophrenia patients, where the excitatory synaptic elements are significantly reduced, while the inhibitory synaptic elements are less affected [64–66]. Hence, our study proposes an alternative non-cell-autonomous mechanism where OPC malfunction suppresses excitatory synaptogenesis thus contributing to pathogenesis of schizophrenia. Both excitatory and inhibitory synapses also connect neurons to OPCs [67, 68], it is tempting to examine whether the neuron-OPC synapses are also implicated in schizophrenia-related pathological changes, which may explain the results of previous imaging studies. Schizophrenia is usually diagnosed between late adolescence and early thirties [53, 54], when myelination is still ongoing [55, 56]. Any abnormal activation of Wnt/β-catenin pathway in OPCs within this age may affect their differentiation and hinder myelination [57, 58]. Indeed, our findings in schizophrenia patients and mouse model show that Wnt-signaling elements in the OPCs are hyperactive especially at the initiation of schizophrenia-like pathology in mice, suggesting that defects in myelination may follow later when disease evolves [9, 10]. Although we still lack direct evidence linking OPC hypertrophies to Wnt-signaling hyperactivation, as well as their association with DISC1-Δ3 splicing in schizophrenia patients, these early changes in OPCs, (found in both schizophrenia patients and mouse model), may either initiate or contribute to the onset of schizophrenia.

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**DISCUSSION**

We identified hypertrophic OPCs as a new histopathological signature of schizophrenic brains in both human patients and mouse model, provided compelling evidence that expression of DISC1 exon 3 splicing variant solely in OPCs is sufficient to suppress synaptogenesis, and presented a potential mechanism by which the onset of schizophrenia related to OPCs could be driven by the overproduction of Wif1, a Wnt-pathway inhibitor, in response to the hyperactivation of Wnt/β-catenin signaling in DISC1-Δ3 OPCs. Evidence gathered previously suggests that myelin defects contribute to pathophysiology of schizophrenia [7]. However, several DTI studies on young patients showed no changes in white matter at the onset of disease [8–11]. This contradicts the current hypothesis postulating that schizophrenic phenotypes originate from myelin defects, and hints at an alternative possibility that myelin abnormalities may not be necessarily responsible for the onset of disease in young schizophrenia patients, but develop as the disease progresses [8–11]. Our in-vivo and in-vitro findings demonstrate that abnormal OPC function initiates neuronal malfunction. In particular, expression of a single gene variant DISC1-Δ3 in the OPCs is sufficient to trigger the onset of schizophrenia-related pathological changes, which may explain the results of previous imaging studies. Schizophrenia is usually diagnosed between late adolescence and early thirties [53, 54], when myelination is still ongoing [55, 56]. Any abnormal activation of Wnt/β-catenin pathway in OPCs within this age may affect their differentiation and hinder myelination [57, 58]. Indeed, our findings in schizophrenia patients and mouse model show that Wnt-signaling elements in the OPCs are hyperactive especially at the initiation of schizophrenia-like pathology in mice, suggesting that defects in myelination may follow later when disease evolves [9, 10]. Although we still lack direct evidence linking OPC hypertrophies to Wnt-signaling hyperactivation, as well as their association with DISC1-Δ3 splicing in schizophrenia patients, these early changes in OPCs, (found in both schizophrenia patients and mouse model), may either initiate or contribute to the onset of schizophrenia.
phosphorylation. By analyzing both direct and indirect regulation, we provided new insights into how DISC1-Δ3 stabilizes β-catenin and promotes Wnt/β-catenin signaling in the OPCs, which may act as a starting point of schizophrenia pathogenesis. This new mechanism is in agreement with observations showing altered Wnt pathway genes such as GSK3β, WIF1, as well as AKT1 in schizophrenia postmortem brains [43, 44, 70]. Notably, manipulating Wif1 production alone improves synaptogenesis and mitigates schizophrenia-like behaviors in DISC-Δ3 mice. Consequently, DISC-Δ3 represents a perspective target for novel treatment strategies. DISC1 can also regulate neurogenesis, neurite outgrowth, and synaptic plasticity, which are all involved in the pathogenesis of schizophrenia [16, 36, 71, 72]. These processes however could also be regulated by many other factors,
and hence the relative contribution of DISC1 requires further studies.

Notably, OPCs hypertrophy is not linked to the overactivation of Wnt/β-catenin pathway in DISC1-Δ3 mice, as our experiments, as well as previous studies, failed to induce such morphological change by increasing Wnt/β-catenin signaling pathway activity. Changes in OPCs morphology may be caused by DISC1 acting as a scaffolding protein, with the DISC1-Δ3 variant changing the cytoskeletal dynamics through AKT/mTOR signaling [36, 73]. Such possibility was supported by our KEGG pathway analysis. Alternatively, prolonged inhibition of GSK3β can cause cell hypertrophy [74]. Be it as it may, morphological changes correlate with the functional changes in OPCs, and thus OPCs hypertrophy may be used as a histological mark for early-stage schizophrenia.

In conclusion, our findings provide new evidence of the pathological potential of OPCs in the onset of schizophrenia in genetically susceptible individuals, whereas targeting WIF1 in OPCs may open a new direction in developing effective therapeutic strategies for schizophrenia.

METHODS

Human schizophrenia tissues and immunohistochemical staining

Human schizophrenia and healthy comparable post-mortem tissue slides were provided by the National Health and Disease Human Brain Tissue Resource Center at Zhejiang University in China (S2019017). All human tissues were collected following fully informed consent by the donors via a tissue bank system. The number of positive cells in the region of interest was counted, and the positive area per cell was calculated as: positive area/number of cells. The Sholl analysis was performed using a Fiji ImageJ plugin described previously [80]. This measurement was only done on pairs in which the cell processes were clearly seen. Quantification of synapse puncta was done using images captured by the V3000 confocal microscope, which was then analyzed using the Fiji software. The fluorescent intensity and Western-blots positive band, the positive areas were automatically selected in image-Pro Plus software. The areas of interest (AOI) were separated by setting the threshold at least two times the background.

Statistical analysis

Statistical significance between groups was determined using the GraphPad Prism software 9.0 (GraphPad Software, San Diego, CA, USA). Data were presented as means ± standard deviation (SD). Fold changes reported in the text are (mean difference ± SEM of difference) % compared to the controls. The unpaired t-test was used to determine the significance between two experimental groups, while one-way analysis of variance (ANOVA) was used to determine statistical significance when comparing multiple groups. All statistical tests were two-tailed. p-values less than 0.05 were considered statistically significant. Significant statistical results are indicated as: *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001. No statistical methods were used to predetermine sample sizes but our sample sizes are similar to those reported in the previous publications [42]. No sample or animal was excluded from the analysis. Transgenic mouse litters were randomly subjected to different experiment paradigm. The investigators were blinded when analyzing the data. Data distribution was assumed to be

neuron spontaneous excitatory postsynaptic currents (sEPSCs) and inhibitory postsynaptic currents (sIPSCs) recording, whole-cell patch-clamp recordings were made with borosilicate glass pipettes. Cells were visualized with infrared optics on an upright microscope (BX51WI, Olympus). A MultiClamp 700B amplifier and pCLAMP10 software were used for electrophysiology (Axon Instruments).

WIF1 shRNA Retrovirus and Stereotaxic surgery

To silence WIF1 expression in vivo, three shRNA sequences targeting different sites of WIF1 mRNA were designed, and a scrambled shRNA target sequence was designed as a negative control. The shRNA sequences were inserted into the previously described retrovirus vector [51]. Using stereotaxic techniques, WIF1 shRNA Retrovirus was injected bilaterally into the hippocampus CA1 of the PI0 mouse. Two days post retrovirus injection, mice were perfused with 4% buffered paraformaldehyde.

Quantification methods

Images captured with the V5200 Research Slide Scanner and V3000 confocal microscope were used to quantify cell numbers. To quantify positive cell numbers, cell counting was conducted on nine randomly chosen fields for each sample using an Image Pro Plus image analysis system. The number of cells in a fixed area in the CA1 region was quantified manually. To quantify the fluorescence positive area per cell, a threshold was set to include the fluorescence positive signal, followed by quantification of the area of positive signals in the region of interest; the number of positive cells in the region of interest was counted, and the positive area per cell was calculated as: positive area/number of cells. The Sholl analysis was performed using a Fiji Sholl Analysis plugin described previously [80]. This measurement was only done on pairs in which the cell processes were clearly seen. Quantification of synapse puncta was done using images captured by the V3000 confocal microscope, which was then analyzed using the Fiji software. The fluorescent intensity and Western-blots positive band, the positive areas were automatically selected in image-Pro Plus software. The areas of interest (AOI) were separated by setting the threshold at least two times the background.
normal, but this was not formally tested. The F-test was conducted to compare the variance between the groups during statistical analysis. All experiments were performed at least 3 times, and the findings were replicated in individual mice and cell cultures in each experiment.

DATA AVAILABILITY
All data are available in the main text or the supplementary materials. RNA-seq data have been deposited in the NCBI GEO under the accession number GSE183341.
24. Nakata K, Lipska BK, Hyde TM, Ye T, Newburn EN, Weinberger DR, et al. Interactions of human truncated DISC1 proteins: implications for schizophrenia. Transl Psychiatry. 2011;1:e30.

25. Provenzano FA, Guo J, Wall MM, Feng X, Sigmon HC, Brucato G, et al. Hippocampal pathology in clinical high-risk patients and the onset of schizophrenia. Biol Psychiatry. 2020;87:234–42.

26. Newburn EN, Hyde TM, Ye T, Morita Y, Weinberger DR, Kleinman JE, et al. Interactions of human truncated DISC1 proteins: implications for schizophrenia. Transl Psychiatry. 2011;1:e30.

27. Gao R, Ponzio P. Common mechanisms of excitatory and inhibitory imbalance in schizophrenia and autism spectrum disorders. Curr Mol Med. 2015;15:146–67.

28. Callicott JH, Straub RE, Pezawas L, Egan MF, Mattay VS, Hariri AR, et al. Variation in DISC1 affects hippocampal structure and function and increases risk for schizophrenia. Nat Genet Sci USA. 2005;102:8627–32.

29. Chen TJ, Kula B, Nagy B, Barzan R, Gall A, Ehrlich I, et al. In vivo regulation of βATF4 in colon cancer cells. Sci Rep. 2018;8:3178.

30. Newburn EN, Hyde TM, Ye T, Morita Y, Weinberger DR, Kleinman JE, et al. Interactions of human truncated DISC1 proteins: implications for schizophrenia. Transl Psychiatry. 2011;1:e30.

31. Nose M, Sawa A, Mortensen PB. Schizophrenia. Lancet. 2016;388:86–97.

32. Vos T, Abajobir AA, Abbafati C, Abbas KM, Abd-Allah F, et al. Global, regional, and national incidence, prevalence, and years lived with disability for 328 diseases and injuries in 195 countries, 1990–2016: a systematic analysis for the Global Burden of Disease Study 2016. Lancet. 2017;390:1211–59.

33. World Health Organization. Schizophrenia. Geneva, 2022.

34. Vos T, Abajobir AA, Abbafati C, Abbas KM, Abd-Allah F, et al. Global, regional, and national incidence, prevalence, and years lived with disability for 328 diseases and injuries in 195 countries, 1990–2016: a systematic analysis for the Global Burden of Disease Study 2016. Lancet. 2017;390:1211–59.

35. Lochhead PA, Kinstrie R, Sibbet G, Rawjee T, Morrice N, Cleghon V. A chaperone-competent GSKbeta transitional intermediate mediates activation-loop autophosphorylation. Mol Cell. 2006;24:2627–33.

36. Maas DA, Valles A, Martens GJM. Oxidative stress, prefrontal cortex hypomyelination and cognitive symptoms in schizophrenia. Transl Psychiatry. 2017;7:e1711.

37. Naqvi NH, Tsuang MT. Oligodendrogial progenitor cell reprogramming and synapse formation. J Neurosci. 2012;31:2697–703.

38. McKeown AM, Millan MJ. G protein-coupled receptors in neuroprotection and neurodegeneration. J Neurosci. 2014;34:11929–47.

39. Millar JK, Wilson-Annan JC, Anderson S, Christie S, Taylor MS, Semple CA, et al. DISC1 association, heterogeneity and interplay in schizophrenia and bipolar disorder. Mol Psychiatry. 2009;14:865–73.

40. Millan MJ, Millan-Munne J, Gil-Abad P, Bhatia S, Doherty G, McKeown AM, et al. G protein-coupled receptors in neuroprotection and neurodegeneration. J Neurosci. 2014;34:11929–47.

41. Mukhopadhyay D, Schubert D, Pringle JR, Charest H, Busch H, Ebert TL, et al. A new secreted protein that binds to Wnt proteins and inhibits their activities. Science. 1995;270:665–7.

42. Nakata K, Lipska BK, Hyde TM, Ye T, Newburn EN, Monti A, et al. DISC1 splice variants are upregulated in schizophrenia and associated with risk polymorphisms. Proc Natl Acad Sci USA. 2009;106:15873–8.

43. Nakata K, Lipska BK, Hyde TM, Ye T, Newburn EN, Monti A, et al. DISC1 splice variants are upregulated in schizophrenia and associated with risk polymorphisms. Proc Natl Acad Sci USA. 2009;106:15873–8.

44. Nakata K, Lipska BK, Hyde TM, Ye T, Newburn EN, Monti A, et al. DISC1 splice variants are upregulated in schizophrenia and associated with risk polymorphisms. Proc Natl Acad Sci USA. 2009;106:15873–8.

45. Nakata K, Lipska BK, Hyde TM, Ye T, Newburn EN, Monti A, et al. DISC1 splice variants are upregulated in schizophrenia and associated with risk polymorphisms. Proc Natl Acad Sci USA. 2009;106:15873–8.

46. Nakata K, Lipska BK, Hyde TM, Ye T, Newburn EN, Monti A, et al. DISC1 splice variants are upregulated in schizophrenia and associated with risk polymorphisms. Proc Natl Acad Sci USA. 2009;106:15873–8.

47. Nakata K, Lipska BK, Hyde TM, Ye T, Newburn EN, Monti A, et al. DISC1 splice variants are upregulated in schizophrenia and associated with risk polymorphisms. Proc Natl Acad Sci USA. 2009;106:15873–8.

48. Nakata K, Lipska BK, Hyde TM, Ye T, Newburn EN, Monti A, et al. DISC1 splice variants are upregulated in schizophrenia and associated with risk polymorphisms. Proc Natl Acad Sci USA. 2009;106:15873–8.

49. Nakata K, Lipska BK, Hyde TM, Ye T, Newburn EN, Monti A, et al. DISC1 splice variants are upregulated in schizophrenia and associated with risk polymorphisms. Proc Natl Acad Sci USA. 2009;106:15873–8.

50. Nakata K, Lipska BK, Hyde TM, Ye T, Newburn EN, Monti A, et al. DISC1 splice variants are upregulated in schizophrenia and associated with risk polymorphisms. Proc Natl Acad Sci USA. 2009;106:15873–8.
54. McGrath J, Saha S, Chant D, Welham J. Schizophrenia: a concise overview of incidence, prevalence, and mortality. Epidemiol Rev. 2008;30:67–76.

55. Grydeland H, Vérites PE, Váša F, Romero-Garcia R, Whitaker K, Alexander-Bloch AF, et al. Waves of maturation and senescence in micro-structural MRI markers of human cortical myelination over the lifespan. Cereb cortex. 2019;29:1369–81.

56. Miller DJ, Duka T, Stimpson CD, Schapiro SJ, Baze WB, McArthur MJ, et al. Prolonged myelination in human neocortical evolution. Proc Natl Acad Sci USA. 2012;109:16480–5.

57. Fancy SP, Baranzini SE, Zhao C, Yuki DI, Irvine KA, Kaiting S, et al. Dysregulation of the Wnt pathway inhibits timely myelination and remyelination in the mammalian CNS. Genes Dev. 2009;23:1571–85.

58. Feigenson K, Reid M, See J, Crenshaw EB 3rd, Grinspan JB. Wnt signaling is sufficient to perturb oligodendrocyte maturation. Mol Cell Neurosci. 2009;42:255–65.

59. Falcao AM, van Bruggen D, Marques S, Meijer M, Jakel S, Agirre E, et al. Disease-specific oligodendrocyte lineage cells arise in multiple sclerosis. Nat Med. 2018;24:1837–44.

60. Kirby L, Jin J, Cardona JG, Smith MD, Martin KA, Wang J, et al. Oligodendrocyte precursor cells present antigen and are cytotoxic targets in inflammatory demyelination. Nat Commun. 2019;10:3887.

61. Brey F, Kloc M, Chavalil M, Hussein I, Wilson M, Christoffel DJ, et al. Genetic and stress-induced loss of NG2 Glia triggers emergence of depressive-like behaviors through reduced secretion of FGFR2. Neuron. 2015;88:941–56.

62. Wang Y, Su Y, Yu G, Wang X, Chen X, Yu B, et al. Reduced oligodendrocyte precursor cell impair astrocytic development in early life stress. Adv Sci. 2021;8:e2001811.

63. McCutcheon RA, Reis Marques T, Howes OD. Schizophrenia—an overview. JAMA Psychiatry. 2020;77:201–10.

64. Eastwood SL, Harrison PJ. Decreased expression of vesicular glutamate transporter 1 and complexin II mRNAs in schizophrenia: further evidence for a synaptic pathology affecting glutamate neurons. Schizophr Res. 2005;73:159–72.

65. Harrison PJ, Eastwood SL. Preferential involvement of excitatory neurons in medial temporal lobe in schizophrenia. Lancet. 1998;352:1669–73.

66. De Rosa A, Fontana A, Nuzzo T, Garofalo M, Di Maio A, Punzo D, et al. Machine Learning algorithm unveils glatamatergic alterations in the post-mortem schizophrenia brain. NPJ Schizophr. 2022;8:8.

67. Moura DMS, Brennan EJ, Brock R, Cossas LA. Neuron to oligodendrocyte precursor cell synapses: protagonists in oligodendroglial development and myelination, and targets for therapeutics. Front Neurosci. 2021;15:779125.

68. Zhang X, Liu Y, Hong X, Li X, Meshul CK, Moore C, et al. NG2 glia-derived GABA release tunes inhibitory synapses and contributes to stress-induced anxiety. Nat Commun. 2021;12:5740.

69. Jensen J, Brennessvik EO, Lai YC, Shepherd PR. GSK-3beta regulation in skeletal muscles by adrenaline and insulin: evidence that PKA and PKB regulate different pools of GSK-3. Cell Signal. 2007;19:204–14.

70. Hallmayer J. Getting our AKT together in schizophrenia? Nat Genet. 2004;36:115–6.

71. Miyoshi K, Honda A, Baba K, Taniguchi M, Oono K, Fujita T, et al. Disrupted-in-Schizophrenia 1, a candidate gene for schizophrenia, participates in neurope outgrowth. Mol Psychiatry. 2003;8:685–94.

72. Tropea D, Hardingham N, Millar K, Fox K. Mechanisms underlying the role of DSC1 in synaptic plasticity. J Physiol. 2018;595:2747–71.

73. Enomoto A, Asai N, Namba T, Wang Y, Kato T, Tanaka M, et al. Roles of disrupted-in-schizophrenia 1-interacting protein girdin in postnatal development of the dentate gyrus. Neuron. 2009;63:774–87.

74. Sugden PH, Fuller SJ, Weiss SC, Clerk A. Glycogen synthase kinase 3 (GSK3) in the heart: a point of integration in hypertrophic signalling and a therapeutic target? A critical analysis. Br J Pharmacol. 2008;153:5137–153.

75. Zhu X, Hill RA, Dietrich D, Komitova M, Suzuki R, Nishiyama A. Age-dependent fate and lineage restriction of single NG2 cells. Development. 2011;138:745–53.

76. Doerflinger NH, Macklin WB, Popko B. Inducible site-specific recombination in myelinating cells. Genes. 2003;35:63–72.

77. Kang SH, Fukaya M, Yang JK, Rothstein JD, Bergles DE. NG2+ CNS glial progenitors remain committed to the oligodendrocyte lineage in postnatal life and following neurodegeneration. Neuron. 2010;68:668–81.

78. Schüller U, Heine VM, Mao J, Kho AT, Dillon AF, Han YG, et al. Acquisition of granule neuron precursor identity is a critical determinant of progenitor cell competence to form Shh-induced medulloblastoma. Cancer Cell. 2008;14:123–34.

79. Robanus-Maandag EC, Koelink PJ, Breukel C, Salvatori DC, Jagmohan-Changur SC, Bosch CA, et al. A new conditional Apc-mutant mouse model for colorectal cancer. Carcinogenesis. 2010;31:946–52.

80. Ferreira TA, Blackman AV, Oyer J, Jayabal S, Chung AJ, Watt AJ, et al. Neuronal morphometry directly from bitmap images. Nat Methods. 2014;11:982–4.

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Conceptualization: JN, LX, and AV Methodology: JN, LX, CY, and YC Investigation: JN, GY, YS, CY, GY, YS, BY, GC, CY, YW, WX, SW, QW, XH, and JN Visualization: JN, GY, YS, CY, GY, YS, BY, and FYD Funding acquisition: JN, LX, CY, and YC Project administration: JN and LX Supervision: JN and LX Writing—original draft: JN, CY, and LX Writing—review & editing: JN, CY, HC, FM, and LX, and AV.

Competing interests

The authors declare no competing interests.

Additional information

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