Altered Subcellular Distribution of the 75-kDa DISC1 Isoform, cAMP Accumulation, and Decreased Neuronal Migration in Schizophrenia and Bipolar Disorder: Implications for Neurodevelopment

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Keywords

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SUMMARY

Background: *DISC1* (Disrupted-In-Schizophrenia-1) is considered a genetic risk factor for schizophrenia (SZ) and bipolar disorder (BD). *DISC1* regulates microtubule stability, migration, and cAMP signaling in mammalian cell lines and mouse brain tissue. cAMP is a regulator of microtubule organization and migration in neurons. Aberrant microtubule organization has been observed in olfactory neuronal precursors (ONP) derived from patients with SZ and BD, which suggests involvement of *DISC1* and cAMP. However, the biology of *DISC1* in the pathophysiology of psychiatric conditions remains elusive. **Aims:** Herein, utilizing ONP obtained from SZ, BD patients and healthy subjects, we have studied *DISC1* expression, protein levels, and subcellular distribution by qRT-PCR, immunoblotting, subcellular fractionation, and confocal microscopy. Cell migration and cAMP accumulation were assessed by Transwell and PKA competition assays. **Results:** We found increased levels of the 75-kDa *DISC1* isoform in total cell extracts of ONP from patients with SZ and BD compared with controls. Subcellular distribution showed a significant decrease of cytoplasmic *DISC1* concomitant with its augmented levels in transcription sites. Moreover, significant cAMP accumulation and diminished migration were also observed in patients' cells. **Conclusion:** Alterations of *DISC1* levels and its cellular distribution, which negatively modify cAMP homeostasis, microtubule organization, and cell migration, in ONP from patients with SZ and BD, suggest that their presence in early stages of brain development may impact brain maturation and function.

Introduction

Schizophrenia (SZ) and bipolar disorder (BD) are two of the most highly heritable chronic mental disorders with a lifetime prevalence of about 1% of the world population. Although different in their clinical presentation, these disorders share psychotic symptoms and cognitive impairment associated with brain structural abnormalities [1–3]. The neurodevelopmental hypothesis proposes that SZ and BD originate in the prenatal period due to impairments in neuronal migration and synaptogenesis, leading to abnormal brain maturation and neuronal connectivity [4–7]. Among others, *DISC1* (Disrupted-In-Schizophrenia-1) has been considered a genetic risk factor for SZ and BD. Microtubule organization and microtubule-associated proteins play a key role in neuronal migration and establishment of connections between neurons. Cytoplasmic *DISC1* interacts with microtubules through their associated proteins MAP1A, NUDEL, LIS1, and M1PT3, modulating microtubule dynamics in

a polymerization-dependent manner [8–10]. Microtubules are important components in the formation of the leading edge process, acting as guides for the nuclear movement into the edge (nucleokinesis) [11,12]. Additionally, these cytoskeleton components facilitate cell retraction enveloping the nucleus in a “cage”-like structure [13,14]. Decreased MAP2 expression has been reported in postmortem brain tissue of patients with SZ [15,16]. On the other hand, *DISC1* is also localized in the nucleus where it interacts with the activating transcription factor 4 (ATF4) [17]. The formed complex ATF4/*DISC1* represses the transcription of the PDE (phosphodiesterase) 4D9 variant resulting in accumulation of cAMP [18], which is an important modulator of microtubule arrays and migration of neurons [19–21]. A postmortem study has shown nuclear enrichment of *DISC1* in the orbitofrontal cortex of patients with SZ [22], while in mammalian cells, nuclear *DISC1* colocalizes with the promyelocytic leukemia (PML) nuclear bodies, which are nuclear compartments for gene transcription [17].

The olfactory neuroepithelium is considered a unique human model to study neurodevelopment as it contains immature neurons and maintains neurogenesis throughout adult life [23,24]. Biopsies of this tissue obtained from psychiatric patients have been used to study the pathophysiology of some of these disorders [25–27]. Recently, we have obtained cells from the olfactory neuroepithelium of healthy control subjects (HS) by exfoliation of the nasal cavity, and their characterization showed the presence of olfactory neuronal precursors (ONP), which we were able to differentiate in culture [28]. Recent studies of the ONP microtubular arrangement of patients with SZ have shown microtubule disorganization and wide areas devoid of these structures associated with alterations in ionic channel modulation. In contrast, ONP derived from patients with BD showed short microtubules, lower levels of total tubulin, and no alteration in voltage-activated Ca^{2+} currents [29]. Although these observations allow a better understanding of the microtubular organization in patient neuronal cells, it is still unknown whether alterations in microtubule components and their functions could impact cellular processes involved in SZ and BD brain development. Thus, in the present study, we used primary cultures of ONP from BD, SZ patients, and HS to characterize molecular mechanisms in which DISC1 might modulate organization and function of the cytoskeleton.

The present results showed increased levels of DISC1 75-kDa isoform in the nuclear cellular fraction of BD and SZ patients' ONP and the protein colocalization in PML nuclear bodies. Significant increase of cAMP levels was also observed in ONP derived from patients as well as lower cytoplasmic levels of the 75-kDa DISC1 isoform concomitant with its nuclear translocation. None of these changes were observed in ONP from HS. Our data suggest that microtubule disorganization and decreased migration of ONP from patients with SZ and BD could result from diminished levels of DISC1 in the cytoplasmic pool and the higher levels of cAMP may reflect transcriptional repression of PDE by nuclear DISC1 presence in transcriptional sites.

Materials and Methods

Subjects

Participants were recruited from the Schizophrenia and Bipolar Disorder Clinics of the Instituto Nacional de Psiquiatría in Mexico City. Diagnoses were established independently by two general psychiatrists specially trained in diagnosing and treating BD or SZ following the clinical criteria of the Diagnostic and Statistical Manual of Mental Disorders—fourth edition revised (DSM-IV R) [30]. Patients were included only when a diagnostic agreement was reached by the two clinicians. All participants provided written informed consent prior to their involvement in the study, which was previously approved by the Institution's ethics committee.

Exfoliates of nasal mucosa were obtained from five patients with SZ, five patients with BD, and five from healthy control subjects (HS) and without any medication. Participants were receiving different types of antipsychotic and mood stabilizers when the cell exfoliation procedure was performed. None were in the first psychotic episode. All were free of allergies and at chronic stages of their clinical conditions, and two in the SZ group were drug naïve (Table S1).

Cell Culture

Cells were exfoliated from the anterior region of the medial lateral turbinate by using a brush of 2.4 cm in length and 3 mm in diameter, brushing in circular movements in the lateral wall of nasal cavity and septum. Cells were harvested in Dulbecco's modified Eagle and F-12 media (DMEM/F-12) supplemented with 10% fetal bovine serum, 4 mM L-glutamine, 100 $\mu\text{g}/\text{mL}$ streptomycin, and 100 IU/mL penicillin. Cells were frozen and stored in liquid nitrogen as described [28]. The experiments were carried out with cells in passages 6–10 cultured in Petri dishes (Nunc, Roskilde, Denmark) or in 24-well multidishes (Corning Costar, Corning, NY, USA). All the results correspond to $n = 5$ for each group.

Immunofluorescence

As previously described, ONP grown on coverslips were processed for immunofluorescence [28]. A rabbit anti-DISC1 antibody (Invitrogen, Carlsbad, CA, USA) and the rabbit anti-PML antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA) were used at 1:50 dilution. Secondary staining was performed by incubation for 1 h at room temperature with FITC- or TRITC-labeled secondary antibodies (Jackson ImmunoResearch Lab, West Grove, PA, USA) (1:100 dilution) directed to the first antibody and then rinsed as indicated before. Nuclei were stained with 20 $\mu\text{g}/\text{mL}$ DAPI (4', 6'-diamidino-2-phenylindole dihydrochloride) for 3 min. Coverslips were mounted on glass slides with Vectashield (Vector Labs, Burlingame, CA, USA) and examined by confocal microscopy (SP2 Leica Microsystems, Wetzlar, Germany). Images were analyzed with the Leica's microscope imaging software (LAS-AF-Lite 2.6.0, Leica Microsystems, Wetzlar, Germany).

Western Blotting

Cells were lysed, sonicated, and centrifuged as previously described by our group [31]. The supernatants were recovered, and the protein concentration was measured using the BCA protein assay (Thermo Scientific, Rockford, IL, USA). Forty micrograms of protein was loaded in 10% polyacrylamide gels and separated by SDS-PAGE. Proteins were electrotransferred onto nitrocellulose membranes (0.45 μm , Bio-Rad, Irvine, CA, USA). Membranes were incubated with the primary antibodies (dilution: DISC1 1:100; GAPDH 1:7000; hnRNP A1 1:200) overnight at 4°C, followed by incubation with peroxidase-conjugated secondary antibodies, and revealed with the ECL Western blotting detection reagent (Amersham). Anti-mouse GAPDH antibody was used as a loading control maker (Millipore, Billerica, MA, USA). Reactive protein bands were quantitatively evaluated by scanning densitometry using the NIH ImageJ software.

Subcellular Fractionation

Subcellular fractionation was performed as previously reported [32]. Briefly, ONP were scraped off the cultured dishes into a hypotonic buffer containing Complete® and then transferred to a microfuge tube. Scraped cells were centrifuged 5 min at $1000 \times g$. The supernatant recovered was the cytoplasmic fraction. The pellet, or nuclear fraction, was rinsed twice with

hypotonic buffer, resuspended in cold buffer C, and incubated in ice for 20 min followed by centrifugation at $16000 \times g$ for 10 min at 4°C to remove cellular debris. Supernatants were considered nuclear fractions. Cytoplasmic and nuclear fractions were electrophoresed using SDS-PAGE in 10% polyacrylamide gels.

PCR Reactions

Total RNA was extracted from ONP with TRIZOL (Life Technologies Inc., Carlsbad, CA, USA). The yield of total RNA was determined by absorbance at 260 nm using a NanoDrop 1000 spectrophotometer (Thermo Scientific). Total RNA (500 ng) was used in 25 μ L reverse transcriptase reactions to synthesize cDNA, using a SuperScript First-Strand Synthesis System for RT-PCR (Invitrogen). The primers used were the following, DISC1: forward 5'-TCCATCACGAGACGAGACTG-3', reverse 5'-TCCCAGCT TTTT GACATTCC-3', RPLP0: forward 5'-CAGATTGGCTACCCAAGT-3', reverse 5'-GGGAAGGTGTAATCCGTCT-3'.

Quantitative Real-time PCR

Relative expression levels of mRNAs were measured by real-time quantitative (RT-PCR) using FastStart SYBR Green Master (Roche, Roche, Germany) in a Real Time 7000 Thermal Cycler (Applied Biosystems, Grand Island, NY, USA). Specific oligonucleotides for DISC1 and housekeeping RPLP0 are listed above. Relative expression levels were calculated by using $2^{-\Delta\Delta C_t}$ equation. Data represent experiments performed in triplicate. The data were normalized to the value of the housekeeping RPLP0 gene.

Cyclic AMP Assay

A method reported by Osorio-Espinoza A et al. [33] was used in this study with minor modifications. In short, suspended cells were incubated with 3 μ M forskolin for 5, 10, 20, and 30 min at 37°C. cAMP was determined by competition assays using 50 μ L samples containing the PKA regulatory subunit (0.5 UI per sample) and [3 H] cAMP (10 nM). After 2.5 h at 4°C, reactions were terminated by filtration over GF/B filters presoaked in 0.3% polyethylenimine. After washing, radioactivity was determined by liquid scintillation counting. The amount of cAMP was calculated by using a standard cAMP curve (10^{-12} – 10^{-5} M).

Transwell Migration Assay

ONP were previously serum-deprived for 12 h, after that time ~80% of the cells were synchronized in G0/G1 phase (data not shown). 3×10^4 ONP resuspended in 200 μ L of serum-free DMEM/F-12 were seeded in the upper chamber of a Transwell (8 μ m pore size, Costar) placed in 24-well plates. The lower chamber was filled with 500 μ L of DMEM/F-12 supplemented with 10% of FBS. After 6 h incubation at 37°C and 10% CO₂, ONP in the upper surface of membranes were gently removed with cotton pads. Cells that migrated through the Transwell membranes were fixed with ice-cold methanol for 5 min at –20°C and stained with DAPI for 3 min. Pictures were taken under an inverted fluorescence microscope employing a 20 $\times$ objective. Ten fields per insert were counted, and the number of cells was determined using Image-Pro Plus software. Experiments were performed in duplicate, and migration was expressed as percentage of the cells that migrated. Data were normalized to the value of HS cells.

Statistical Analysis

Comparisons between groups were made with one-way or two-way analyses of variance (ANOVA) and Dunnett's post-test. Data are expressed as mean $\pm$ SEM. Statistical analysis was performed using GraphPad Prism software version 5.0c. Statistical differences were considered significant as $P < 0.05$.

Results

Basic clinical and sociodemographic data of all participants are shown in Table S1. There were no significant age differences between the groups (mean $\pm$ SD), HS: 35.0 ± 11.75 ; SZ: 29.80 ± 7.91 ; BD: 31.60 ± 12.05 ; $P > 0.05$. Only two of the five patients with a schizophrenia diagnosis included in this study were drug-naïve.

In the three groups, expression levels of DISC1 mRNA were measured by RT-PCR using total mRNA obtained from the ONP and the primers listed in Methods. In Figure 1, PCR products of DISC1 in the three groups studied are presented. A single band was observed in all of them, which corresponds to DISC1 sequence from exon 6 to exon 9. RPLP0 was used as a control for gene expression. No differences were found in the level of intensity of the PCR products between the three groups (Figure 1A).

Figure 1 DISC1 mRNA expression in olfactory neuronal precursors (ONP) obtained from patients with SZ and BD was not altered in comparison with healthy control subjects (HS) (A) PCR products of DISC1 (570 bp fragment) and the housekeeping gene RPLP0 (98 bp fragment) in ONP from SZ, BD patients and HS (B) DISC1 mRNA relative expression in ONP normalized to RPLP0 levels. Values represent mean $\pm$ SEM ($n = 5$). Data were expressed as a percentage of DISC1 mRNA expression levels of HS olfactory neuronal precursors; no statistical differences were observed between groups, $P > 0.05$.

Quantitative RT-PCR analysis confirmed the results obtained by endpoint RT-PCR (Figure 1B). No statistical differences were observed between groups.

DISC1 protein expression was analyzed by Western blot using an anti-DISC1 antibody directed to its C-terminal domain, although this antibody should recognize the full-length DISC1

protein (100 kDa). Figure 2A shows that the antibody only recognized a major band at 75 kDa in HeLa and neuroblastoma SH-SY5Y cell extracts [22]. This antibody also revealed a 75-kDa band in the HS and patients' ONP (Figure 2B). The lack of the 100-kDa isoform in Western blots of the ONP extracts suggests higher liability of the N-terminal domain of the 100-kDa isoform as proposed

Figure 2 DISC1 protein is overexpressed in olfactory neuronal precursors (ONP) from patients with SZ and BD. (A) Characterization of the antibody against the C-terminal domain of DISC1 in SH-SY5Y and HeLa. A major band of approximately 75 kDa was detected. (B) Representative Western blot showing 75-kDa DISC1 isoform and the load control band protein in whole cell extracts of ONP obtained from SZ, BD patients and HS. (C) Relative abundance of DISC1 in olfactory neuronal precursors from SZ, BD patients and HS were analyzed in the Western blot by densitometry using as loading control GAPDH. The scatter graph represents the mean $\pm$ SEM obtained in three independent experiments. All the densitometry data were normalized to the relative band intensity of DISC1 obtained from the ONP of HS ($n = 5$; *** $P < 0.0001$).

Figure 3 Lower levels of DISC1 in the cytoplasm of olfactory neuronal precursors (ONP) obtained from patients with SZ and BD. (A) Representative confocal images of ONP from SZ, BD patients and healthy control subjects (HS). DISC1 (red) was stained with TRITC, and nuclei (blue) were stained with DAPI, Bar = 40 μ m. Higher immunoreactivity in the nuclei (insets, right column) and lower in the cytoplasm (circles, left column) of DISC1 protein are observed in ONP of patients with SZ and BD compared to healthy control subjects' (HS) ONP (B) Representative Western blot of cytoplasmic (c) and nuclear (n) fractions of DISC1 obtained from ONP of SZ, BD patients and HS. DISC1 levels in the cytoplasm and the nucleus were normalized to GAPDH and hnRNPA1 expression levels. (C) Cytoplasmic (c) to nuclear (n) ratio of DISC1 protein content in NP obtained from SZ, BD patients and HS (mean $\pm$ SEM). All the densitometry data were normalized to the relative band intensity of DISC1 obtained from the neuronal precursors of HS. Patient's cells had significantly lower levels of DISC1 in the cytoplasmic fraction ($n = 5$; *** $P < 0.0001$).

by other authors [22,34]. A robust increase in the expression of the 75-kDa band was observed in total cell extracts of patients' ONP. GAPDH in cell extracts was used as loading control and revealed with an anti-GAPDH antibody. Densitometric analysis from three independent experiments showed a significant increase of DISC1 75-kDa isoform of nearly twofold in both the SZ and the BD group in comparison with values of the HS group (normalized to value = 1) (Figure 2C). Results demonstrated that there was no correlation between DISC1 mRNA expression and protein levels.

DISC1 subcellular distribution was studied in ONP by immunofluorescence and confocal microscopy. In Figure 3A, DISC1 immunoreactivity in both the nucleus and the cytoplasm is observed. A clear enrichment of DISC1 protein in the nuclei (insets, right column) concomitant with a cytoplasmic decrease (circles, left column) is observed in ONP of patients with SZ and BD compared to cells from the HS group. Figure 3B shows a representative immunoblot of the nuclear and cytoplasmic fractions obtained from HS and patients' ONP using the anti-DISC1 antibody. The same blots after stripping were incubated with specific antibodies for proteins only present in the cytoplasmic (GAPDH) or in nuclear fractions (hnRNP A1). Results indicated that the fractions were not cross-contaminated. These data confirm the nuclear enrichment of DISC1 visualized by immunofluorescence in ONP obtained from patients with SZ and BD. The blots also show that a small fraction of the DISC1 isoform is localized in the cytoplasm. The cytoplasmic/nuclear ratio of DISC1 was calculated, and results are shown in Figure 3C. The results showed a signifi-

cant decrease (more than 50%) in the cytoplasmic pool of DISC1 in ONP of patients with SZ and BD.

Previous studies have shown that transfected DISC1 into HeLa cells is colocalized with PML nuclear bodies [17]. To characterize the nuclear localization of DISC1 in ONP, a simultaneous staining with anti-PML and anti-DISC1 antibodies was performed. The results in Figure 4 show that both antibodies stained the nuclei (confirmed with the use of DAPI staining) in a punctuate pattern in ONP of the three groups studied, colocalization of these two proteins is clearly observed in the right columns (yellow color), indicating that nuclear DISC1 is associated with nuclear transcription domains (PML nuclear bodies).

cAMP content was measured in ONP challenged with forskolin to promote the accumulation of this nucleotide through stimulation of the adenylate cyclase pathway [35]. Figure 5 shows the kinetics of cAMP production. The graphs indicate that cAMP levels in ONP of HS are maintained constant in the presence of forskolin. In contrast, cAMP levels in ONP from patients gradually increased to reach a significant accumulation at 30 min of forskolin stimulation.

As cytoplasmic DISC1 interaction with microtubule-associated proteins plays a key role in microtubular dynamics and neuronal migration, this process was measured using Transwell migration assays in ONP of the three groups of individuals studied. Figure 6 shows representative immunofluorescence images of patient and healthy control subjects ONP stained with anti- β -III-tubulin antibody. ONP of HS (left panel) showed typical organization of microtubules in parallel arrays that extended to the cell edges

Figure 4 Nuclear DISC1 is localized in the promyelocytic leukemia (PML) nuclear bodies. Representative confocal (z-axis slide) images of olfactory neuronal precursors obtained from SZ, BD patients and healthy control subjects (HS) expressing DISC1 and PML. Immunofluorescent staining showed that DISC1 (red) colocalized with PML (green). The merge showed in yellow the colocalization (arrowheads, right columns), Bar = 7.5 μ m.

Figure 5 Forskolin induces cAMP accumulation in olfactory neuronal precursors (ONP) from patients with SZ and BD. cAMP was measured in ONP from SZ, BD patients and HS after 5, 10, 20, and 30 min cells interaction in 3 μ M forskolin. cAMP levels produced in basal conditions were subtracted from cAMP values determined in presence of forskolin. The scatter graph shows the means $\pm$ SEM measured in duplicates of two independent experiments of each condition. ONP obtained from HS did not show changes in cAMP concentration during the presence of the forskolin stimulus (A). Neuronal precursors from SZ (B) and BD (C) patients show an accumulation at 30 min after the forskolin presence ($n = 5$; $*P < 0.05$).

Figure 6 Microtubular organization is altered, and migration is diminished in olfactory neuronal precursors (ONP) of patients with SZ and BD. (A) Representative immunofluorescent images of ONP obtained from SZ, BD patients and HS stained with anti-III β -tubulin antibody. Arrowheads point the discontinuous microtubular patterns, bar = 20 μ m. (B) The histogram represents the percentage of cells per field that migrate through the Transwell membrane. Data are expressed as means $\pm$ SEM of three independent experiments and were normalized to HS group values ($**P < 0.001$, $***P < 0.0001$).

without interruptions. In contrast, in ONP derived from patients with SZ and BD (middle and right panels), disorganized microtubules do not extend into the cells leading processes (arrowheads). When migration was analyzed in ONP, the percentage of cells that migrated toward the fetal bovine serum stimulus was significantly lower (~20%) in cells from patients than in HS cells (Figure 6B), showing correlation between diminished cytoplasmic levels of DISC1 and microtubule alterations.

Discussion

Human population genetic studies from different parts of the world have proposed that *DISC1* is a risk factor for SZ and BD [34,36–38]. However, it is still unknown how DISC1 protein could

participate in the etiology of these diseases. The use of primary cultures of human ONP in this study has several advantages over studies that use postmortem brain tissue and established cell lines. Among these, dissociated cells maintained in primary cultures are not transformed by multiple passages as the established cell lines and retain their biological properties present in their natural location [39–41]. Primary cultures of human ONP have been previously characterized as immature neurons due to the presence of β -III-tubulin and nestin, for their ability to differentiate *in vitro* and to maintain the function of calcium channel currents and structural properties up to passage 10 [28]. Furthermore, these cells reflect developing gene expression profiles reported in studies of molecular changes associated with brain diseases [42]. Therefore, primary cultures of ONP offer the possibility to characterize

biomarkers and to implement routine laboratory test for diagnosis. This model allows performance of pharmacologic screening to implement new pharmacological therapies useful for treatment of neuropsychiatric diseases. In our study, the use of primary cultures of ONP derived from the olfactory epithelium of patients with SZ and BD has provided clear advantages for characterization of DISC1 in an immature neuron population.

Results obtained showed no differences in the parameters studied regarding the type of medication administered in both groups of patients (Table S1). In ONP of patients with SZ and BD, there was no correlation between *DISC1* expression and the total protein levels that were augmented, suggesting that *DISC1* mRNA could be stable. Also, an increased half-life of DISC1 may occur due to defective proteasome degradation. Although the data obtained here do not disclose the mechanisms for increased levels of DISC1 to occur in ONP patients, our analysis has shown distinct differences between subcellular levels of the protein in the cytoplasmic pool and the nuclei.

In human postmortem brain tissue, a slight increase in the expression of the 75-kDa DISC1 isoform was associated with SZ but not with BD [22,43]. Using ONP from patients with SZ and BD, we have found a noticeable increase in the levels of the 75-kDa DISC1 isoform. Discrepancy in our results with those reporting lack of DISC1 overexpression in postmortem brain tissue may be explained by several confounding factors, including postmortem interval, antemortem medication, tissue quality, as well as the considerable cellular heterogeneity in brain tissue.

In addition, using ONP derived from patients with SZ and BD has allowed to envision diminished levels of cytoplasmic DISC1; and its interaction with microtubule-associated proteins [8,10] could result in the altered microtubular organization previously reported in these psychiatric conditions [29].

Radial and tangential migrations of cortical neurons at embryonic stages are regulated by DISC1 [44,45], and pharmacological disruption of microtubules abolishes this cellular process [46]. In this study, we showed that decreased migration in ONP of patients is associated with microtubule disorganization, in particular, a

scarce microtubular network in the leading edge and an altered microtubular nuclear cage. This defective organization of microtubules may interfere with nucleokinesis and cell retraction necessary to generate forward cell movements. These observations and the accumulation of cAMP in ONP from patients with SZ and BD suggest that decreased migration of ONP from patients is related to these alterations as they were not observed in ONP from HS.

The relationship between cAMP accumulation and decreased migration was validated in ONP derived from patients by our experiments (data not shown), utilizing the neuronal chemoattractant BDNF [47]. Furthermore, as already described [19], our data indicated that stimulation of ONP migration was inhibited by forskolin. In fact, it is known that antipsychotic drugs block cAMP synthesis through dopamine systems [48,49], supporting that alterations in DISC1 trigger defective pleiotropic interactions and changes in the structural organization of microtubules that result in decreased migration of ONP.

In conclusion, our present results with human immature neurons (ONP) from patients with SZ and BD showed common alterations in DISC1 levels and its cellular distribution, cAMP homeostasis, and cell migration that were not observed in HS. These aberrant processes may hinder normal neurodevelopment and favor progression to disease.

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Conflict of Interests

The authors declare no conflict of interest.

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Supporting Information

The following supplementary material is available for this article:

Table S1. Clinical and Demographic Data of Subjects.