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Dysbindin-1 contributes to prefrontal cortical dendritic arbor pathology in schizophrenia

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Abstract

Deep layer III pyramidal cells in the dorsolateral prefrontal cortex (DLPFC) from subjects with schizophrenia and bipolar disorder previously were shown to exhibit dendritic arbor pathology. This study sought to determine whether *MARCKS*, its regulatory protein dysbindin-1, and two proteins, identified using microarray data, *CDC42BPA* and *ARHGEF6*, were associated with dendritic arbor pathology in the DLPFC from schizophrenia and bipolar disorder subjects. Using western blotting, relative protein expression was assessed in the DLPFC (BA 46) grey matter from subjects with schizophrenia (n=19), bipolar disorder (n=17) and unaffected control subjects (n=19). Protein expression data were then correlated with dendritic parameter data obtained previously. *MARCKS* and dysbindin-1a expression levels did not differ among the three groups. Dysbindin-1b expression was 26% higher in schizophrenia subjects (p=0.01) and correlated inversely with basilar dendrite length (r=−0.31, p=0.048) and the number of spines per basilar dendrite (r=−0.31, p=0.048), but not with dendritic spine density (r=−0.16, p=0.32). The protein expression of *CDC42BPA* was 33% higher in schizophrenia subjects (p=0.03) but, did not correlate with any dendritic parameter (p>0.05). *ARHGEF6* 87 kDa isoform expression did not differ among the groups. *CDC42BPA* expression was not altered in frontal cortex from rats chronically administered haloperidol or clozapine. Dysbindin-1b appears to play a role in dendritic arbor pathology observed previously in the DLPFC in schizophrenia.

1.1 Introduction

Both schizophrenia (SZ) and bipolar disorder (BD) are chronic mental illnesses associated with high disability. Worldwide, SZ accounts for 7.4% and BD for 7% of disability-adjusted life years lost (DALYs) (Whiteford et al., 2013). Clinically, SZ is classified as a psychotic disorder whereas BD is categorized as a mood disorder. However, genetic (Cross-Disorder Group of the Psychiatric Genomics Consortium, 2013), imaging (Ellison-Wright and Bullmore, 2010), neuropsychological and neurophysiological (Clementz et al., 2016) data

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suggest that SZ and BD are more related biologically than previously appreciated. Indeed, we demonstrated that pyramidal cells in the deep layer III of the dorsolateral prefrontal cortex (DLPFC) in both SZ and BD subjects exhibit similar dendritic arbor pathology (Konopaske et al., 2014).

Neuronal cytoskeletal structures are central to the generation, maintenance, morphology and function of dendrites and their processes (Newey et al., 2005). Extracellular signals modulate the activity of three small Rho GTPases, *RHOA*, *RAC1* and *CDC42*, which in turn, activate signaling cascades to influence the actin cytoskeleton and microtubules. Rho GTPases are active in their GTP-bound state and inactive when GDP-bound. The GTP/GDP ratio and regulatory molecules influence Rho GTPase activity. Guanine nucleotide exchange factors (GEFs) enhance the replacement of GDP by GTP increasing GTPase activity. GTPase activating proteins (GAPs) promote GTP hydrolysis and inhibit GTPase activity. Lastly, guanine nucleotide dissociation inhibitors (GDIs) inhibit GTPase activity by precluding the exchange of GDP for GTP (Newey et al., 2005).

Using microarray data generated from the DLPFC obtained from a cohort of SZ, BD and control subjects, we identified several genes, known to regulate the actin cytoskeleton, whose transcripts were differentially regulated in SZ subjects (Konopaske et al., 2015). Included in this list was the gene, *MARCKS*, a PKC substrate which regulates the actin cytoskeleton and dendritic arbor (Li et al., 2008). Previously, we demonstrated that the mRNA expression of *MARCKS* was increased in the DLPFC from SZ and BD subjects (Konopaske et al., 2015). Although *MARCKS* mRNA expression did not correlate with dendritic parameters, it remained possible that its relative protein expression level might be associated with dendritic pathology. Dysbindin-1 protein expression was also examined in the current study since it regulates *MARCKS* expression (Okuda et al., 2010) and is coded by *DTNBP1*, which some studies have suggested might be a SZ and BD risk gene (Breen et al., 2006; Fallin et al., 2005; Gaysina et al., 2009; Joo et al., 2007; Straub et al., 2002; Van Den Bogaert et al., 2003). However, it should be noted that *DTNBP1* was not implicated as a SZ risk gene in a very large genome-wide association study (GWAS) (Schizophrenia Working Group of the Psychiatric Genomics, 2014).

The chief objectives of the current study were to determine if the relative protein expressions of *MARCKS* and dysbindin-1 correlated with dendritic arbor pathology in the DLPFC from subjects with SZ and BD. In addition, we analyzed the protein expression of two candidate genes identified along with *MARCKS* based on microarray data as being differentially expressed in the DLPFC from subjects with SZ: *ARHGEF6* and *CDC42BPA* (Konopaske et al., 2015). *ARHGEF6* is a RhoGEF associated with X-linked mental retardation (Kutsche et al., 2000). *ARHGEF6* is expressed in dendrites and spines and regulates dendrite growth (Node-Langlois et al., 2006; Ramakers et al., 2012; Totaro et al., 2012). *CDC42BPA* is an effector protein of *CDC42* and *RAC1*. It has homologies with *ROCK* and is involved in dendritogenesis (Chen et al., 1999). Since dendritic arbor pathology might be a significant component to the pathophysiology of SZ and BD (Konopaske et al., 2014), the identification of relevant proteins could lead to novel diagnostic and/or therapeutic interventions.

2.1 Experimental/Materials and Methods

Protein Candidate Identification

To identify candidate actin regulating proteins which might be involved in dendritic pathology in both SZ and BD, we assessed previously obtained microarray data. The microarray data was generated from DLPFC tissue from SZ (n=19), BD (n=18), and control (n=25) subjects. Tissue processing and data analysis methods have been described previously (Konopaske et al., 2015; Konradi et al., 2004). In the current study, we examined the relative protein expression of *MARCKS* (Konopaske et al., 2015; Pinner et al., 2014), dysbindin-1 (Tang et al., 2009a), *CDC42BPA* (CDC42 binding protein kinase alpha), and *ARHGEF6* (αPIX).

2.2 Tissue Processing

Frozen, postmortem, human brain tissue samples containing DLPFC (BA 46) were obtained from the Harvard Brain Tissue Resource Center (McLean Hospital, Belmont, MA). The cohort (the McLean 75 cohort) included SZ (n=19), BD (n=17), and control (n=19) subjects, which were matched as closely as possible for age, sex, and postmortem interval (PMI). Diagnoses were based on a review of medical records and a questionnaire completed by the donor's family and were made using Feighner criteria for SZ (Feighner et al., 1972) and DSM-III-R criteria (American Psychiatric Association, 1987) for BD. Each brain was examined by a neuropathologist for gross and microscopic changes associated with neurodegenerative disorders, cerebrovascular disease, infectious processes, trauma or tumors. Clinical and demographic subject data are summarized in Table 1 and Supplementary Table 1.

2.3 Western Blotting

Western blotting methods have been described in detail previously (Balu and Coyle, 2011). Total protein was extracted, Halt protease and phosphatase inhibitor cocktails (Fisher Scientific, Waltham, MA) were added and total protein concentration was quantified. Subjects from each group were loaded in parallel lanes in each gel (e.g., Con, BD, SZ) and samples were separated on 4–20% Mini-Protein TGX gels (Bio-Rad, Hercules, CA), then transferred to PVDF membranes. Membranes were blocked and probed with primary antibodies. See Table 2 for primary antibody data and blocking solutions. Membranes were then incubated with one of the following horseradish peroxidase-conjugated secondary antibodies diluted in the same solution utilized for the primary antibody: goat anti-rabbit (1:5000), goat anti-mouse (1:1500) or donkey anti-goat (1:5000, all from Life Technologies, Carlsbad, CA). Immunocomplexes were visualized using Western Lightning Plus ECL (Perkin Elmer, Waltham, MA). Band images were captured by computerized densitometry using the ChemiDoc XRS+ System (Bio-Rad, Hercules, CA). Captured band image analysis was conducted using Image Lab software (Bio-Rad, Hercules, CA). Analyzed band images were within the linear range of detection and had no evidence of saturation indicated by Image Lab software. Using the volume tool within Image Lab, signal intensity values for captured band images of interest were divided by their corresponding *GAPDH* values. The SZ and BD values were normalized to control values (% control) collected in parallel from the same gel. The normalized values were used for statistical analysis.

2.4 Primary Antibody Selection

For *MARCKS*, a monoclonal mouse antibody used previously in a postmortem SZ study was utilized (Pinner et al., 2014). It produced a band measured at 81 kDa which is very close to that detected in the previous study (e.g., 80 kDa). The antibody for dysbindin-1 was selected using the Human Protein Atlas Database (https://www.proteinatlas.org/, last accessed 04/04/2018). The antibody is a polyclonal rabbit antibody shown to be specific for human dysbindin-1 protein (https://www.proteinatlas.org/, last accessed 04/04/2018). The antibody has been used in a study to detect human dysbindin-1 protein (Gokhale et al., 2015). The measured bands at 52 and 40 kDa were similar to those expected at 55 and 36 kDa. The antibody for *ARHGEF6*, a polyclonal rabbit antibody, was selected using the Human Protein Atlas Database and has been shown to be specific for human *ARHGEF6* protein (https://www.proteinatlas.org/, last accessed 04/04/2018). The antibody has been used to detect human *ARHGEF6* protein in a prior study (Kortum et al., 2015). The measured band at 87 kDa was similar to that expected at 87.5 kDa. Lastly, for *CDC42BPA* a polyclonal rabbit antibody was used. It has been used to detect human *CDC42BPA* protein in a prior study (Zhu et al., 2015b) and the measured band of 197 kDa was similar to that expected at 187 kDa. For further antibody details see Table 2.

2.5 Statistical Analyses

An optimal statistical model was utilized for each protein or protein isoform analyzed. Each of the following factors were systematically assessed alone and in combination with other factors using ANCOVA models with diagnosis included in each model. The factors included age, sex, postmortem interval (PMI; hours), storage time (months), and pH. An optimized model was selected for each protein or protein isoform using the corrected Akaike's Information Criterion (AIC_C) (Akaike, 1973; Hurvich and Tsai, 1989). To control for potential experiment-wise errors, a test-wise false-positive error rate was set at 0.05. Regression diagnostics were run on ANCOVA models to assess for violations of normality, linearity, homoscedasticity and collinearity (Kleinbaum et al., 2012). Post-hoc pairwise comparisons were conducted using Dunnett's method to control for multiple comparisons for any protein or protein isoform having a significant ANCOVA effect for diagnosis ($p < 0.05$). Statistical analyses were conducted using STATA (v. 12, College Station, TX).

Each protein or protein isoform found to have differential relative expression in SZ or BD was assessed for a relationship between its relative expression and basilar dendrite parameters for pyramidal cells having their somata in the deep half of layer III in the DLPFC, obtained previously (Konopaske et al., 2014). Most of the SZ (n=14), BD (n=12), and control (n=15) subjects had available dendrite parameters for analysis. A Pearson correlation coefficient was calculated for the relative expression of each protein or protein isoform of interest and the number of spines per basilar dendrite, basilar spine density, and basilar dendrite length across all subjects.

2.6 Chronic Antipsychotic Administration in Rats

The relative expression of proteins or protein isoforms having differential relative expression in SZ and/or bipolar subjects were analyzed in antipsychotic-administered rats using western blotting. Total protein was extracted from the frontal cortex from rats administered

haloperidol 1mg/kg/day, clozapine 25mg/kg/day, or sterile saline (n=8 per group) i.p. for 28 days. Relative protein expression was assessed by western blotting using methods similar to those for human postmortem tissue.

3.1 Results

Western Blotting

The ANCOVA model utilized for each protein or protein isoform is given in Table 3. No inter-group differences for age, postmortem interval (PMI), storage time, or pH were detected by ANOVA. Except for *MARCKS*, all protein expression data required a log transformation for analysis by ANCOVA due to skewed data distributions. Relative to controls, *MARCKS* protein expression did not differ among SZ, bipolar or control subjects (SZ: 101.6±18.8, BD: 105.2±11.1, Con: 100±9.7; $p>0.05$). For *MARCKS* protein expression, one SZ subject appeared to be an outlier, however, exclusion of the subject did not affect the outcome ($p>0.05$, see Figure 1).

The relative expression of dysbindin-1a did not differ among SZ, BD and control subjects (SZ: 128.5±65.3, BD: 135.7±69.3, Con: 100±26.3; $p>0.05$). Dysbindin-1b expression differed significantly among SZ, bipolar and control subjects ($p=0.02$) with its expression being significantly higher by 26% in SZ vs. control subjects ($p=0.01$). Dysbindin-1b expression was 31% higher in BD subjects, but this did not achieve statistical significance ($p=0.19$; SZ: 125.7±46.4, BD: 130.5±50, Con: 100±20.3, see Figure 1). The protein expression levels the *ARHGEF6* 75 kDa isoform could not be reliably quantified in the full study, so it was not analyzed. The expression level of *ARHGEF6* 87 kDa isoform did not differ significantly among groups (SZ: 116.7±53.4, BD: 106.3±44.8, Con: 100±23.8; $p>0.05$). CDC42BPA expression differed significantly among SZ, bipolar and control subjects ($p=0.02$) with its expression being significantly higher by 33% in SZ vs. control subjects ($p=0.03$). *CDC42BPA* was 10% lower in bipolar subjects, but this was not significant (SZ: 133.1±64.6, BD: 90.3±31.5, Con: 100±51.8; BD vs. con: $p=0.97$; see Figure 2).

The effects of the following clinical variables among SZ and BD subjects on the relative protein expression of dysbindin-1b and *CDC42BPA* were assessed: 1) on antipsychotic medications in the last year of life (yes/no); 2) on lithium at death (yes/no); 3) on valproic acid at death (yes/no); and 4) death by suicide (yes/no). In this cohort, 1/19 SZ and 4/17 BD subjects were off antipsychotic medications in the last year of life. There was no statistical difference in dysbindin-1b or *CDC42BPA* protein expression among BD who were on vs. off antipsychotics in the last year of life ($p>0.05$). 3/19 SZ and 6/17 BD subjects were on lithium at the time death. In addition, 6/19 SZ and 7/17 BD subjects were on valproic acid at the time death. There were no statistical differences in dysbindin-1b or *CDC42BPA* protein expression in either group among subjects on vs. off either lithium or valproic acid at the time of death ($p>0.05$). Lastly, 1/18 SZ subjects and 4/17 BD subjects died by suicide. Death by suicide produced no difference statistically in dysbindin-1b or *CDC42BPA* protein expression among BD subjects ($p>0.05$).

3.2 Correlation with Dendritic Parameters

Across all subjects, dysbindin-1b correlated inversely with basilar dendrite length (r=−0.31, p=0.048) and the number of spines per basilar dendrite (r=−0.31, p=0.048), but not with dendritic spine density (r=−0.16, p=0.32). *CDC42BPA* protein expression did not correlate with any dendritic parameter across all subjects (p>0.05, see Figure 3).

3.3 Chronic Antipsychotic Administration in Rats

No inter-group differences were detected for the relative protein expression of *Cdc42bpa* in the frontal cortex from rats administered haloperidol or clozapine for 28 days (see Figure 4). Dysbindin-1b could not be assessed since it is not expressed in rats (Talbot et al., 2009).

4.1 Discussion

In this western blot study utilizing postmortem human brain tissue from subjects with SZ, BD and controls, the main finding was the relative protein expression of dysbindin-1b was increased in SZ subjects and inversely correlated with basilar dendrite length and the number of spines per basilar dendrite on deep layer III pyramidal cells in the DLPFC. *CDC42BPA* protein expression was increased in SZ subjects, but this did not correlate with dendritic parameters. Thus, dysbindin-1b appears to play a role in dendritic arbor pathology reported previously in the DLPFC in SZ (Glantz and Lewis, 2000; Konopaske et al., 2014).

DTNBP1 codes for dysbindin-1 and is found on chromosome 6p22.3. In primates, there are 3 dysbindin-1 isoforms: 1a, 1b, and 1c. Dysbindin-1 is ubiquitously expressed in human and rodent tissues with dysbindin-1a being the most strongly and widely expressed isoform. Dysbindin-1a is the full-length isoform whereas dysbindin-1b is a truncated version of 1a (Benson et al., 2001; Talbot et al., 2009). Dysbindin-1 is found in the cytoplasm and nucleus and is expressed in axons and dendrites. Large layer III and V pyramidal cells in the neocortex express high concentrations of dysbindin-1. It is associated with the post-synaptic density and microtubules and regulates the morphology of dendritic spines and arbor (Ito et al., 2010; Jia et al., 2014; Talbot et al., 2006; Talbot et al., 2009). Dysbindin-1 is a member of biogenesis of lysosome-related organelles complex 1 (BLOC-1), a ubiquitously expressed protein complex involved in the generation of organelles for the endosomal lysosome system (Starcevic and Dell'Angelica, 2004). BLOC-1 regulates presynaptic membrane trafficking, vesicle generation, membrane fusion mediated by SNARE and pertinent to SZ, glutamate release (Schubert et al., 2012).

In addition to dendritic morphology, dysbindin-1 is involved in several processes with relevance to SZ including cognition, glutamate neurotransmission and dopamine neurotransmission (Talbot et al., 2009). Along with decreased hippocampal spine density, dysbindin-1 knockout mice exhibit impairments in memory and learning (Glen et al., 2014; Jentsch et al., 2009; Jia et al., 2013). Dysbindin-1 regulates glutamate release, calcium signaling and synaptic vesicle pool size (Chen et al., 2008; Saggu et al., 2013). Dysbindin-1 also regulates NMDA signaling, likely by modulating the expression of NMDA receptor subunits (Glen et al., 2014; Karlsgodt et al., 2011; Larimore et al., 2014; Tang et al., 2009b). Moreover, dysbindin-1 controls dopamine neurotransmission by inhibiting dopamine release

and modulating D2 receptor expression (Iizuka et al., 2007; Ji et al., 2009; Jia et al., 2013; Kumamoto et al., 2006).

The mechanism by which dysbindin-1b might lead to dendritic pathology in SZ could be related to its ability to form neurotoxic aggresomes which are cytoplasmic inclusion bodies formed in response to misfolded or excess protein (Xu et al., 2015; Yang et al., 2016; Zhu et al., 2015a). Dysbindin-1b overexpression might cause BLOC-1 components to aggregate and cease functioning (Yang et al., 2016). BLOC-1 inactivity could result in a loss of Arp2/3 regulation which, in turn, might contribute directly to dendritic pathology in SZ (Gokhale et al., 2016).

DTNBP1 was first identified as a SZ risk gene by Straub et al. (2002), but subsequent association studies have been mixed (Talbot et al., 2009). For example, *DTNBP1* was not identified in the large SZ GWAS published in 2014 (Schizophrenia Working Group of the Psychiatric Genomics, 2014). It is through alternate splicing of *DTNBP1* pre-mRNA which results in three dysbindin-1 protein isoforms 1a, 1b, and 1c. A potential SZ risk allele, rs117610176-C, has been associated with SZ and results in increased *DTNBP1b* mRNA transcription (Xu et al., 2015). Thus, areas for future research would be to determine whether the rs117610176-C allele confers increased risk for SZ and if SZ subjects carrying the allele have increased dysbindin-1b protein expression, dysbindin-1b immunoreactive aggresomes and dendritic arbor pathology in the DLPFC.

A prior postmortem study in the DLPFC using in situ hybridization showed reduced dysbindin-1 mRNA in SZ subjects (Weickert et al., 2004). Another study found increased dysbindin-1a and -1b mRNA with no change in dysbindin-1c mRNA utilizing qRT-PCR in the DLFPC in SZ (Tang et al., 2009a). In addition, the same study found reductions in dysbindin-1c with no change in dysbindin-1a or -1b protein expression. The difference in findings between Tang et al. (2009) and the present study likely reflects the relatively small subject numbers included in each study (n=28 & 28 vs. n=19 & 17 & 17). Although *DTNBP1* may not be a SZ risk gene, dysbindin-1 participates in several pathways relevant to the pathophysiology of SZ.

This study found no difference among the groups for the expression level of *MARCKS*. This finding is in contrast to a prior study which reported decreased *MARCKS* in addition to a decrease in is phosphorylated form, *pMARCKS*, in the DLPFC from subjects with SZ (Pinner et al., 2014). A potential reason for the difference between the two studies is that the subjects in Pinner et al. (2014) were considerably older (mean ages: SZ: 78.1±10.5, Con: 77.1±10.4) relative to the current study (mean ages: SZ: 59.5±12.7, BD: 64.2±18.3, Con: 55.8±12.3). In our subjects, there was a small to medium non-significant inverse correlation between age and *MARCKS* expression across all subjects ($r=-0.25$, $p=0.06$). Moreover, age was included in the ANCOVA model for *MARCKS* as determined by AICc. Thus, it is possible that age accounts, at least in part, for the difference between Pinner et al. (2014) and the current study. Another factor which might contribute to the discrepancy between the Pinner et al (2014) and the current study, is the relatively small cohorts (e.g. n=19 in the current study and n=16 in Pinner et al. (2014)). A study having a much larger cohort would likely be revealing and have sufficient power to assess the effects of demographic and

clinical variables on DLPFC *MARCKS* protein expression in both disorders. Despite these discrepancies, it remains possible that *MARCKS* might play a role in the pathophysiology of both SZ and BD.

CDC42BPA is a downstream effector protein of *CDC42* and *RAC1* and regulates neurite outgrowth and filopodia formation (Chen et al., 1999; Leung et al., 1998; Nakamura et al., 2000; Wilkinson et al., 2005). It has homologies with *ROCK* and phosphorylates ERM (ezrin-radixin-moesin) proteins in dendrites, which link the cytoskeleton to membranes (Nakamura et al., 2000). Although *CDC42BPA* protein expression did not correlate with dendritic parameters, as a downstream effector of *CDC42*, it could have indirect effects on dendritic pathology in SZ (Datta et al., 2015; Kuramoto et al., 2009)

As in any study using postmortem brain tissue from SZ subjects, it is critical to consider the potential effects of antipsychotic medication treatment. Since the majority of SZ and BD subjects (18/19 SZ and 13/17 BD subjects) in the current cohort were on antipsychotics in the last year of life, statistical comparisons could not be done among SZ subjects and were not reliable among BD subjects. The effects of chronic antipsychotic administration on protein expression were analyzed in rat frontal cortex. The protein expression of *CDC42BPA* did not differ in the frontal from rats treated for 28 days with haloperidol or clozapine versus controls. A search of the Stanley Neuropathology Consortium Integrative Database (http://sncid.stanleyresearch.org/, last accessed 04/04/2018) was conducted for data on the effects of antipsychotic exposure on *CDC42BPA* in humans with no results. Unfortunately, dysbindin-1b could not be assessed in antipsychotic administered rats since they do not express this isoform. Previously, DLPFC dysbindin-1 protein expression did not correlate with chlorpromazine dose equivalents a month prior to death in SZ subjects and chronic haloperidol administration had no effect on total dysbindin-1 protein expression in the dentate from mice (Talbot et al., 2004; Tang et al., 2009a). Thus, although the effects of antipsychotic medications cannot be ruled out, the probability that the current results for dysbindin-1b and *CDC42BPA* are due to antipsychotics is low.

Data also have also been published assessing the effects of antipsychotics on the expression of the other proteins analyzed in this study. A 9 month administration of haloperidol did not affect expression of *MARCKS* in rat frontal lobe (Pinner et al., 2014). Using LC-mass spectroscopy, Chan et al. (2011) found an inverse correlation between fluphenazine dose equivalents and *MARCKS* protein expression in the DLPFC from SZ and BD subjects. SZ subjects with low antipsychotic exposure and BD subjects not on antipsychotics had increased *MARCKS* protein expression (Chan et al., 2011). Thus, the lack of reduced *MARCKS* expression in the SZ subjects in the present study might reflect, at least in part, antipsychotic treatment. No published data were found on the effects of antipsychotics on *ARHGEF6* protein expression. Moreover, a search of the Stanley Neuropathology Consortium Integrative Database (http://sncid.stanleyresearch.org/, last accessed 04/04/2018) was conducted for data on the effects of antipsychotic exposure on *ARHGEF6* in humans with no results.

The main limitation of the current study is its relatively small number of subjects which might have decreased the study's power to detect intergroup differences. However, an

important strength of the current study is that the results of the relative protein expression analyses could be correlated with previously obtained basilar dendrite parameters for most of the subjects. Another potential limitation of the current study is that the groups were not well balanced for sex. 6/19 SZ and 5/19 control subjects were female, but 16/19 bipolar subjects were female. Sex was included as a potential covariate during the ANCOVA model building procedures to control for this issue. However, despite these statistical controls, this lack of sex balance might have contributed to the differences observed between the present and prior studies and might have influenced any difference detected between diagnostic groups.

When performing protein quantification in human postmortem brain tissue, PMI must be taken into account since proteins degrade at different rates which might affect their quantitation. Although the effects of PMI on protein stability were not analyzed directly in this study, PMI was included as a potential covariate in the ANCOVA model building procedure and none of the analyzed proteins previously exhibited significant degradation in a study of rat brain with PMIs up to 72 hours as analyzed by MALDI-MS (Fountoulakis et al., 2001). Moreover, PMI did not differ significantly between groups making differential protein degradation a source of inter-group difference unlikely.

In conclusion, this study suggests that at least a subset of subjects with SZ have increased expression of dysbindin-1b protein that might contribute to pyramidal cell dendritic arbor pathology in the DLPFC. Subjects with BD also have similar dendritic arbor pathology in the DLPFC, however, dysbindin-1b does not appear to play a role suggesting that different pathophysiological mechanisms occur in SZ and BD producing similar alterations in neuronal morphology. In addition, this study has implicated an effector protein of *CDC42*, *CDC42BPA*, which also might have relevance to dendritic pathology. Further elucidation of the biology of dysbindin-1 in general, and dysbindin-1b in particular, appears warranted especially focusing on polymorphisms regulating its expression and participation in signaling pathways relevant to the pathophysiology of SZ.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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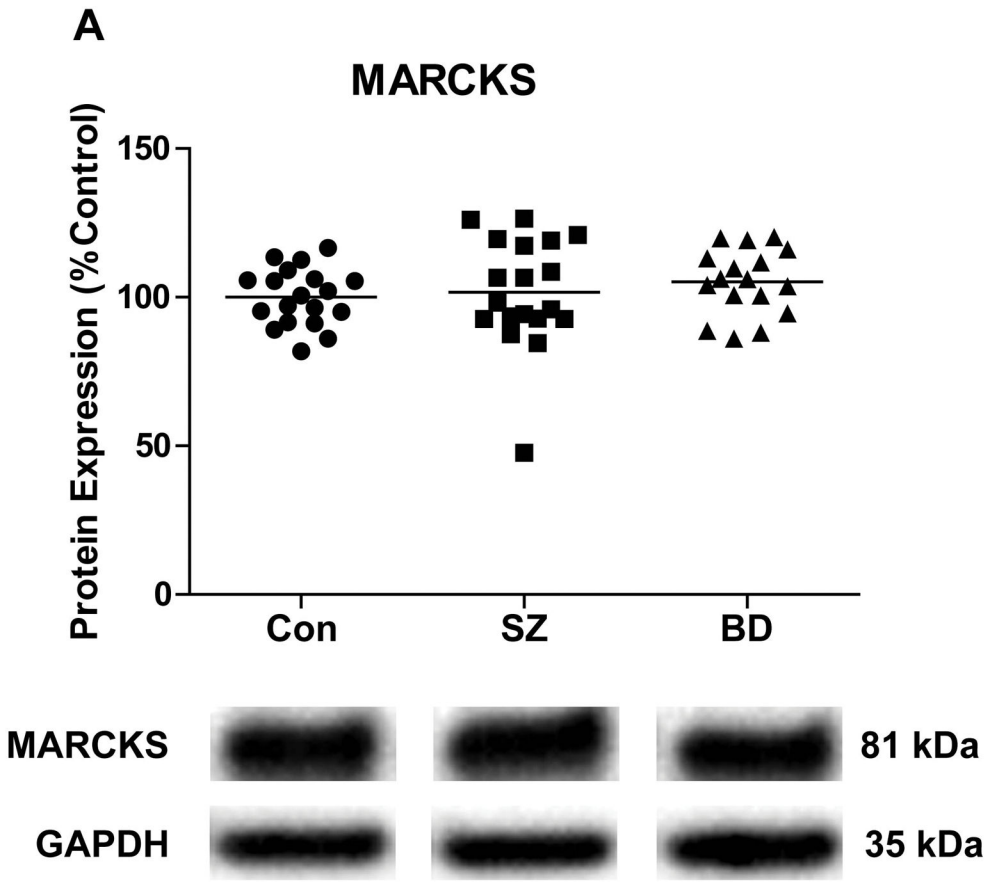


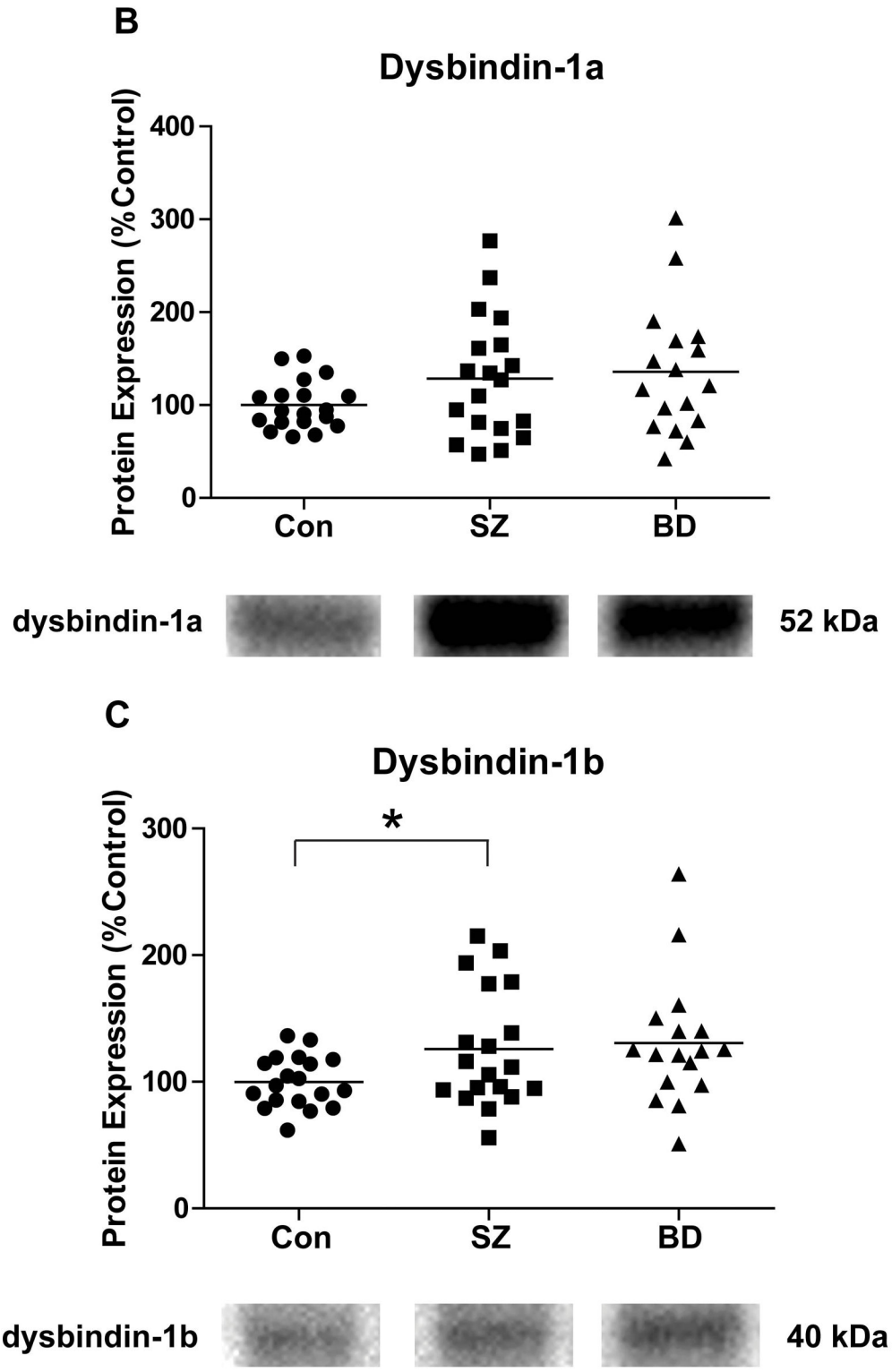


Figure 1.

Graphs depicting the relative protein expression of MARCKS (panel A), dysbindin-1a (panel B) and dysbindin-1b (panel C) in the DLPFC from subjects with schizophrenia (SZ),

bipolar disorder (BD) and controls (Con) assessed using western blotting. Dysbindin-1b expression was significantly increased in schizophrenia subjects relative to controls. No differences were detected between groups for *MARCKS* or dysbindin-1a. * represents $p<0.05$.

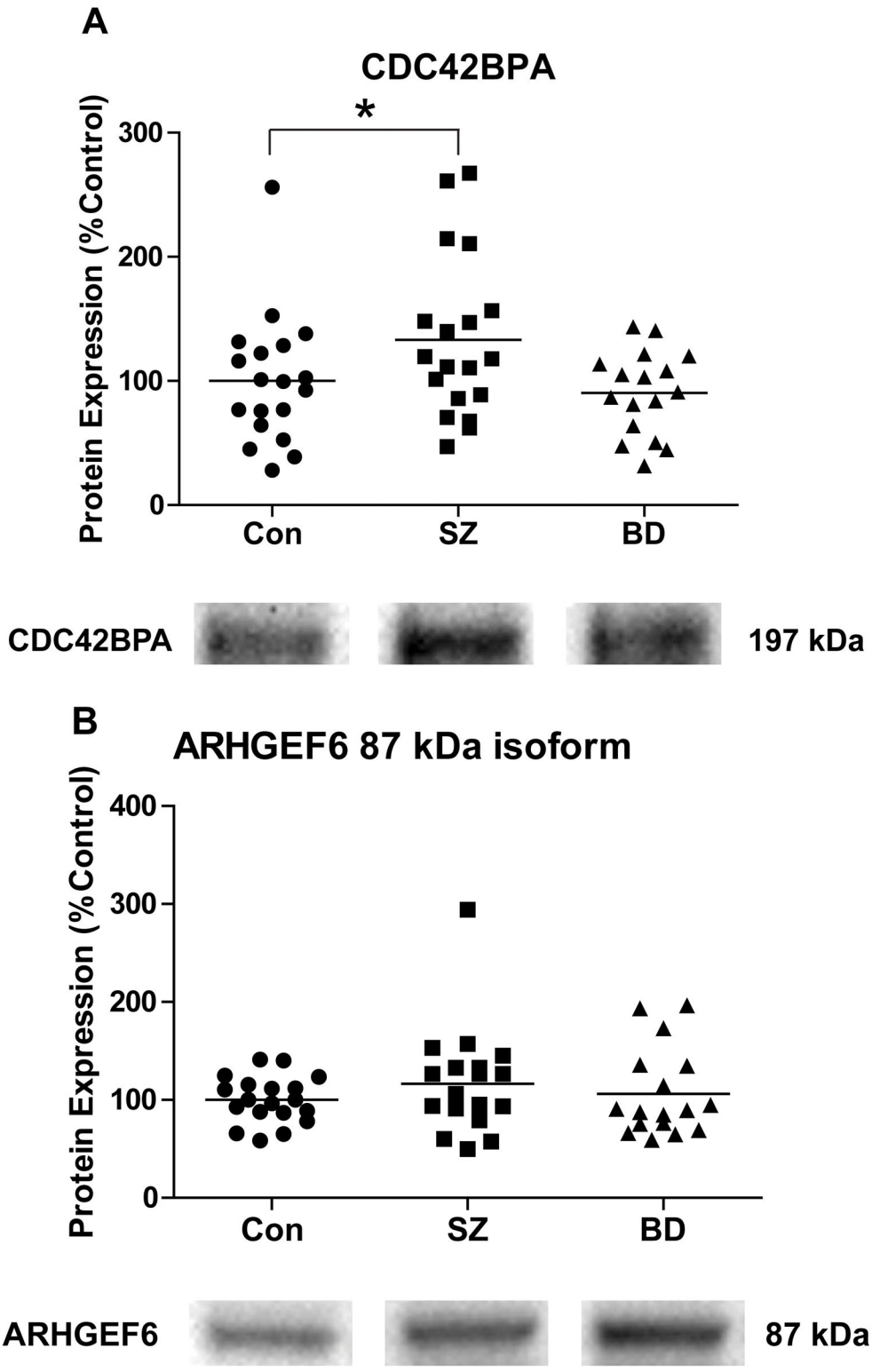
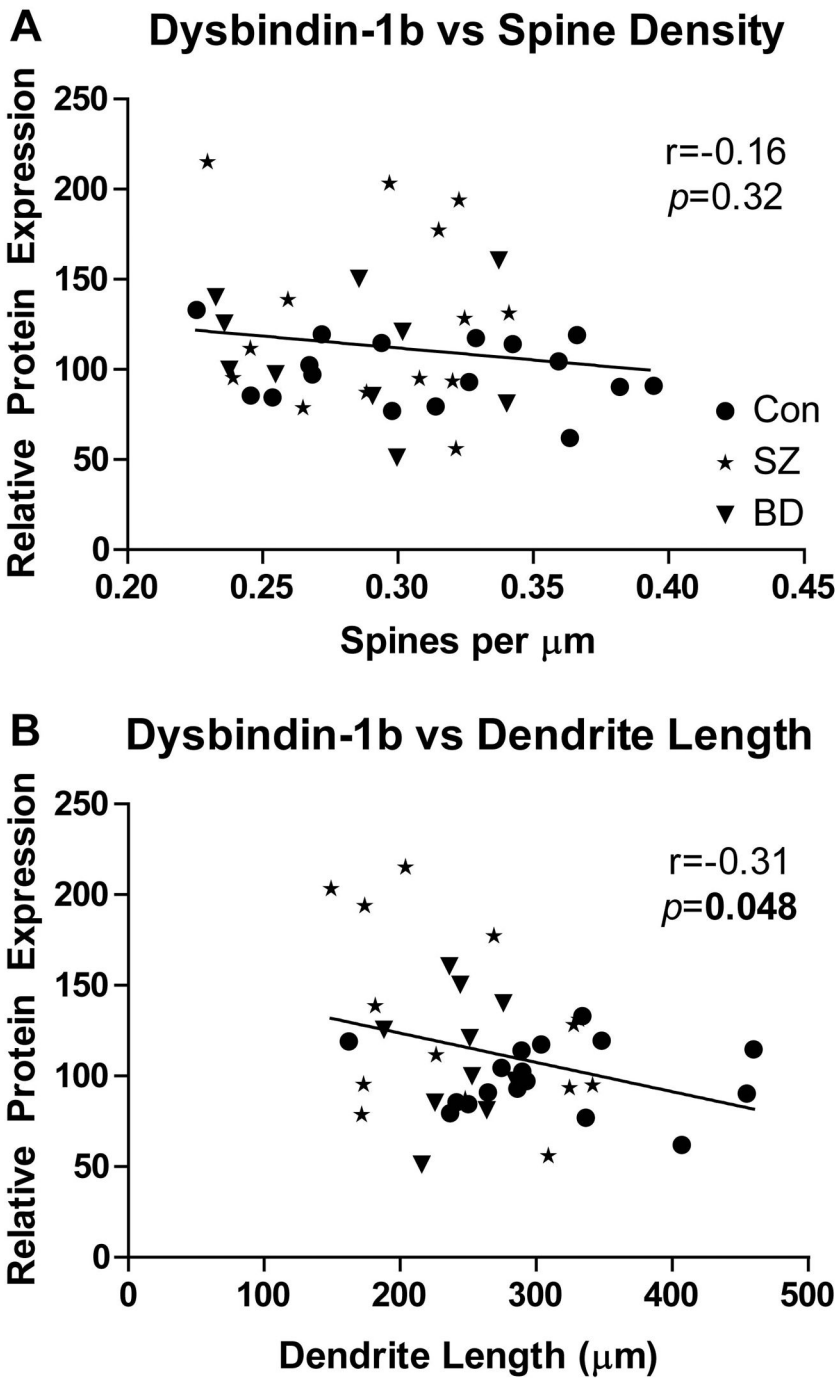
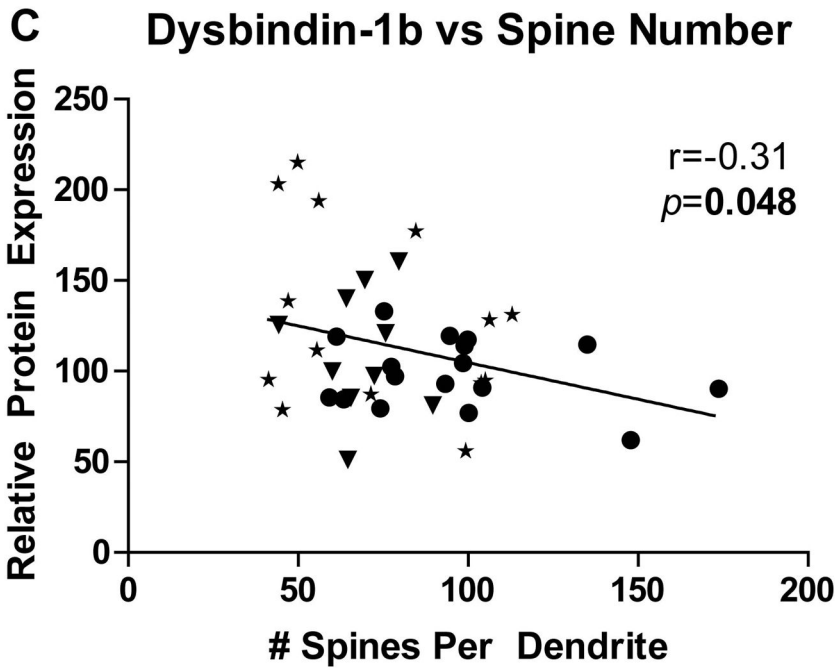


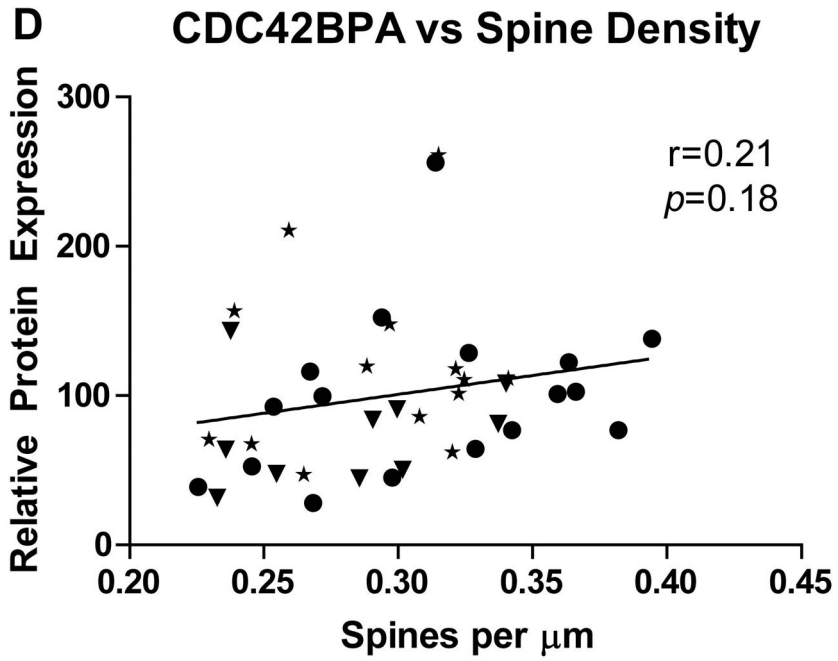
Figure 2.

Graphs depicting the results of western blot analyses for the relative expression of *CDC42BPA* (panel A), and *ARHGEF6* 87 kDa isoform (panel B) in the DLPFC from

subjects with schizophrenia (SZ), bipolar disorder (BD) and controls (Con). *CDC42BPA* expression was significantly increased in schizophrenia subjects relative to controls. No differences were detected between groups for ARHGEF6 87 kDa isoform. * represents $p<0.05$.







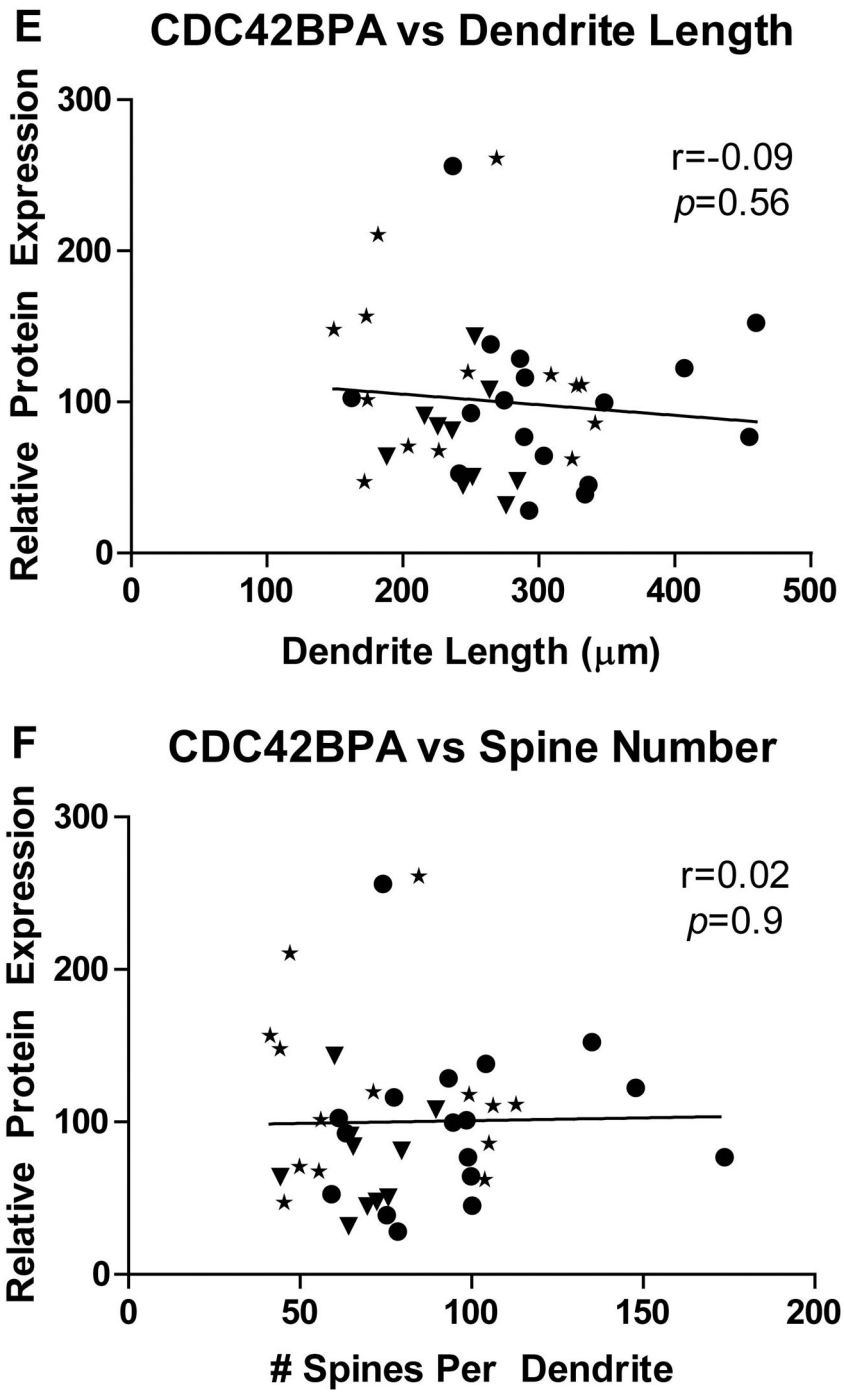


Figure 3.

Plots of relative protein expression levels for dysbindin-1b (panels A, B and C) and CDC42BPA (panels D, E and F) versus basilar dendrite parameters assessed previously for deep layer III pyramidal cells localized in the DLPFC (Konopaske et al., 2014). A Pearson correlation coefficient was calculated across all subjects. The dendritic parameters include spine density (panels A and D), dendrite length (panels B and E) and the number of spines per basilar dendrite (panels C and F). Dysbindin-1b correlated inversely with basilar dendrite

length and the number of spines per basilar dendrite. Control subjects are represented by circles, SZ subjects stars and BD subjects triangles.

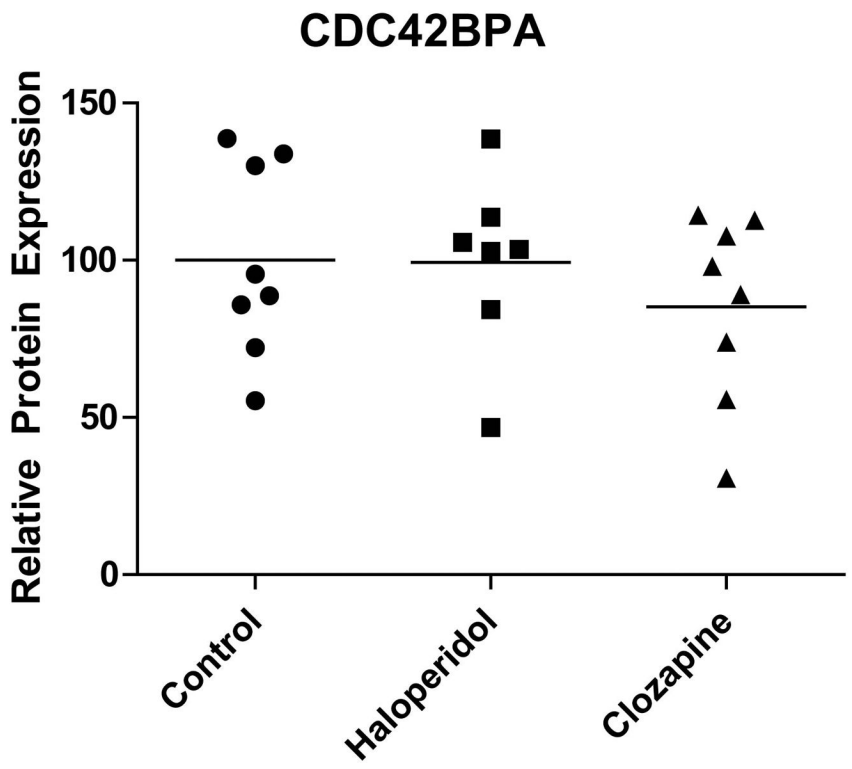


Figure 4.

Graph representing the relative protein expression of *Cdc42bpa* in the frontal cortex of rats chronically administered haloperidol and clozapine. No differences between groups were detected ($p>0.05$).

Table 1

Summary of clinical and demographic data

	Schizophrenia	Bipolar	Control	*p*-value
Sex (M/F)	13/6	3/14	14/5	
Age	59.5±12.7	64.2±18.3	55.8±12.3	*n.s.*
PMI (hours)	23.9±6.6	20.9±6.5	22.5±3.9	*n.s.*
Storage time (months)	137.3±30.5	124.5±28.7	119.7±18.6	*n.s.*
pH	6.5±0.3	6.3±0.3	6.4±0.2	*n.s.*
Suicide (Y/N)	1/18	4/13	0/19	
Antipsychotic medication (Y/N)[1]	18/1	13/4	0/19	
Valproic acid (Y/N)[2]	6/13	7/10	0/19	
Lithium (Y/N)[2]	3/16	6/11	0/19	

[1] in last year of life,

[2] at time of death

Table 2

Primary antibody and blocking solution specifications.

Protein	Antibody Number	Company	Host	Type	Protein loaded (μg)	1° antibody concentration	Blocking solution	Antibody solution[1]
MARCKS	AT2802A	Abgent	Mouse	Monoclonal	10	1:10,000	5% milk	5% BSA[2]
dysbindin-1	HPA029616	Atlas	Rabbit	Polyclonal	20	1:100	5% milk	5% milk
CDC42BPA[3]	AB96659	Abcam	Rabbit	Polyclonal	30	1:100	5% milk	5% BSA
ARHGEF6	AB184569	Abcam	Rabbit	Polyclonal	30	1:125	5% milk	5% BSA
GAPDH	MAB374	Millipore	Mouse	Monoclonal		1:50,000	5% milk	5% BSA
GAPDH[3]	NB300-327	Novus	Rabbit	Polyclonal		1:50,000	5% milk	5% BSA

[1] primary and secondary antibodies were diluted using the same solution;

[2] BSA=bovine serum albumin,

[3] antibody used for both human and rat tissue

Table 3

Summary of relative protein expression ANCOVA models.

Protein	Main Effect	Covariates
MARCKS	diagnosis	PMI, age
dysbindin-1a	diagnosis	pH, sex, PMI, age
dysbindin-1b	diagnosis	pH, age, storage time, sex
CDC42BPA	diagnosis	storage time
ARHGEF6-87	diagnosis	storage time

Note: models were selected using Akaike's Information Criterion (AIC_C).