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Altered prefrontal cortical *MARCKS* and *PPP1R9A* mRNA expression in schizophrenia and bipolar disorder☆

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ABSTRACT

Background: We previously observed dendritic spine loss in the dorsolateral prefrontal cortex (DLPFC) from schizophrenia and bipolar disorder subjects. In the current study, we sought to determine if the mRNA expression of genes known to regulate the actin cytoskeleton and spines correlated with spine loss.
Methods: Five candidate genes were identified using previously obtained microarray data from the DLPFC from schizophrenia and control subjects. The relative mRNA expression of the genes linked to dendritic spine growth and function, i.e. *IGF1R*, *MARCKS*, *PPP1R9A*, *PTPRF*, and *ARHGEF2*, was assessed using quantitative real-time PCR (qRT-PCR) in the DLPFC from a second cohort including schizophrenia, bipolar disorder, and control subjects. Functional pathway analysis was conducted to determine which actin cytoskeleton-regulatory pathways the genes of interest interact with.
Results: MARCKS mRNA expression was increased in both schizophrenia and bipolar disorder subjects. *PPP1R9A* mRNA expression was increased in bipolar disorder subjects. For *IGF1R*, mRNA expression did not differ significantly among groups; however, it did show a significant, negative correlation with dendrite length. *MARCKS* and *PPP1R9A* mRNA expression did not correlate with spine loss, but they interact with NMDA receptor signaling pathways that regulate the actin cytoskeleton and spines.
Conclusions: MARCKS and *PPP1R9A* might contribute to spine loss in schizophrenia and bipolar disorder through their interactions, possibly indirect ones, with NMDA signaling pathways that regulate spine structure and function.

1. Introduction

Spines are small protrusions emanating from dendrites of neurons. Most excitatory neurotransmission in the brain occurs at synapses on spines. Thus, spines are central to a myriad of brain functions (Sekino et al., 2007). Reflecting a possible common pathophysiological feature, we recently demonstrated spine loss in the dorsolateral prefrontal cortex (DLPFC) from both schizophrenia (SZ) and bipolar disorder (BD) subjects (Konopaske et al., 2014), suggesting that spine pathology might contribute to clinical features in both disorders. Indeed, alterations in DLPFC activity have been linked to working memory deficits in SZ (Carter et al., 1998; Manoach et al., 2000; Perlstein et al., 2001; Potkin et al., 2009) and emotional processing in BD (Yurgelun-Todd et al., 2000; Chang et al., 2004; Lawrence et al., 2004; Hassel et al., 2008).

Spines are highly dynamic and the actin cytoskeleton plays a pivotal role in their formation, structure, and maintenance (Sekino et al., 2007). Actin exists as either a globular (G-actin) or filamentous (F-actin) form. G-actin is polymerized to form F-actin, which is a major component of the actin cytoskeleton (Sekino et al., 2007; Shirao and Gonzalez-Billault, 2013). The actin cytoskeleton is also high dynamic and regulated by a host of factors, particularly those related to synaptic transmission.

We sought to identify actin cytoskeleton regulatory genes and assess whether their mRNA expression is altered and correlated with spine loss in the DLPFC of SZ and BD subjects (Konopaske et al., 2014). This study was conducted in three stages. First, we identified actin cytoskeleton regulatory genes using previously obtained microarray data. Second, the relative mRNA expression levels for the genes-of-interest were assessed using quantitative real-time PCR (qRT-PCR). We determined if changes in relative mRNA expression correlated with spine loss. For genes having differential mRNA expression in SZ and/or BD, we determined which actin cytoskeleton-regulating pathway(s) the genes of interest interact with using functional analyses. We postulated that the mRNA expression of one or more genes involved in actin cytoskeleton

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Table 1
Summary of clinical and demographic data for subjects included in the functional analyses (McLean 66 cohort).

	Schizophrenia	Bipolar disorder	Control	*p*-Value
Sex (M/F)	13/6	11/7	17/8	
Age	61.7 ± 18	56.3 ± 18.4	57.6 ± 17	*n.s.*
PMI (hours)	21.6 ± 5.6	21.1 ± 9.7	21 ± 5.4	*n.s.*
pH	6.4 ± 0.3	6.5 ± 0.2	6.4 ± 0.3	*n.s.*
18/28 s rRNA ratio (total RNA)	1.1 ± 0.5	1 ± 0.3	1.1 ± 0.4	*n.s.*

regulation is altered in the DLPFC of subjects with SZ and/or BD and these changes will correlate with the spine loss previously reported (Konopaske et al., 2014).

2. Experiment/materials and methods

2.1. Stage 1: identification of actin cytoskeleton regulatory genes

To identify genes that regulate the actin cytoskeleton and spines in SZ subjects, we analyzed gene expression data from the National Brain Databank: Brain Tissue Gene Expression Repository (Harvard Brain Tissue Resource Center, HBTRC, McLean Hospital, Belmont, MA). The database contains gene expression data for the DLPFC (BA 9) of subjects in the McLean 66 cohort, which consists of SZ (n = 19), BD (n = 18), and controls (n = 25) matched for age, postmortem interval (PMI), gender, pH, and RNA quality (see Table 1). Tissue processing and mRNA extraction procedures have been described previously (Konradi et al., 2004). The gene expression data were produced using Affymetrix (UH-133A) oligonucleotide microarrays for each case and were analyzed using dChip software (Li and Wong, 2001). A false discovery rate (FDR) was implemented to control for multiple tests. Genes having significantly altered expression levels in SZ subjects relative to controls (FDR-corrected $p < 0.05$) were further analyzed using GenMAPP (Gladstone Institutes, University of California at San Francisco) and Ingenuity Pathway Analysis (IPA, Qiagen, Redwood City, CA) (Benes et al., 2004; Konradi et al., 2004) to identify actin cytoskeleton regulatory genes. Our analyses identified 37 potential genes. Since some genes might regulate the actin cytoskeleton, but not spines, the literature pertaining to the function of these 37 genes was reviewed. Based on this review, we selected 5 genes for verification with qRT-PCR, namely, *IGF1R*, *MARCKS*, *PPP1R9A*, *PTPRF*, and *ARHGEF2* (see Table 2) (Cheng et al., 2003; Zito et al., 2004; Calabrese and Halpain, 2005; Ryan et al., 2005).

2.2. Stage 2: qRT-PCR analyses

2.2.1. qRT-PCR subjects

Since frozen DLPFC tissue from the McLean 66 cohort is unavailable, tissue from a second cohort, the McLean 75 cohort, was utilized for the qRT-PCR verification study. Frozen, postmortem, human brain tissue samples containing DLPFC (BA 46) were obtained from the HBTRC (McLean Hospital, Belmont, MA). The cohort included subjects with SZ (n = 22), BD (n = 18), and controls (n = 18), which were matched as closely as possible for age, sex, and PMI. Diagnoses were made using Feighner criteria for SZ (Feighner et al., 1972), and DSM-III-R (American Psychiatric Association 1987) for BD, and were based on a review of medical records and a questionnaire completed by the donor's family. Each brain was examined by a neuropathologist for gross and microscopic changes associated with neurodegenerative disorders, cerebrovascular disease, infectious processes, trauma or tumors.

2.2.2. qRT-PCR tissue processing

Frozen tissue (~100 mg) including all cortical layers and a small portion of white matter was collected into TRIzol (Life Technologies, Grand Island, NY). The tissue was homogenized and total RNA was extracted and purified using the RNeasy Mini Kit (Qiagen, Valencia, CA) according to the manufacturer's instructions. In-column DNase I digestion was carried out to remove the genomic DNA contamination. Total RNA concentration was measured using a Nanodrop 1000 spectrophotometer (Thermo Scientific, Waltham, MA) and RNA integrity was quantified using an Agilent 2100 Bioanalyzer (RNA 6000 Nano Kit, Agilent Technologies, Santa Clara, CA). Only samples having a RNA integrity number (RIN) > 3 were included. cDNA was made using a high capacity cDNA reverse transcription kit (Applied Biosystems, Grand Island, NY).

2.2.3. qRT-PCR analyses

Samples from SZ, BD, and control subjects (n = 8 per group) were used to analyze 6 commonly used housekeeping genes: *PPIA*, *ACTB*, *GAPDH*, *B2M*, *TFRC*, *RPLP0*, and *PGK1*. Results from geNorm analyses (qbasePLUS, Biogazelle, Zwijnaarde, Belgium) revealed that *GAPDH* and *ACTB* expression had the least variance across the three groups. However, *ACTB* had considerable variation among BD subjects: therefore *GAPDH* alone was used as a housekeeping gene. The relative mRNA expression levels of *IGF1R*, *MARCKS*, *PPP1R9A*, *PTPRF*, and *ARHGEF2* were measured in triplicate with qRT-PCR using TaqMan Gene Expression assays (Applied Biosystems, Grand Island, NY) on a Chromo4 Continuous Fluorescence Detection System (Bio-Rad, Hercules, CA). Relative expression levels were calculated using the $2^{-\Delta C_T}$ method.

2.3. Stage 3: functional pathway analyses

The interaction networks of genes, found to have differential mRNA expression in SZ and/or BD by qRT-PCR, were analyzed using IPA and revealed that these molecules participate in a host of signaling pathways. The most relevant pathways are the N-methyl-D-aspartate (NMDA)-type glutamate receptor signaling pathways which modulate actin cytoskeletal organization and in turn, spine structure and function (Halpain et al., 1998; Maletic-Savatic et al., 1999; Fukazawa et al., 2003; Okamoto et al., 2004). Based on the existing literature (Dillon and Goda, 2005; Tada and Sheng, 2006; Cingolani and Goda, 2008) and using IPA, signaling pathways linking NMDA receptors and the actin cytoskeleton in spines were constructed. The genes of interest were then added to the network to further elucidate their interactions with the NMDA receptor

Table 2
Genes identified in Stage 1 for analysis by qRT-PCR.

Gene	Name(s)	Effect(s) on the actin cytoskeleton	Citations
IGF1R	Insulin-like growth factor 1 receptor	Modulates cytoskeleton reorganization	Kim and Feldman (1998)
MARCKS	Myristoylated alanine-rich protein kinase C substrate	F-actin aggregation, filament cross-linking and anchoring to plasma membrane	(Hartwig et al. (1992); Calabrese and Halpain (2005); Li et al. (2008); Yamaguchi et al. (2009))
PPP1R9A	Neurabin-I, protein phosphatase 1, regulatory (inhibitor) subunit 9A	Participates in F-actin polymerization, cross-linking, and cytoskeleton reorganization	(Nakanishi et al. (1997); Oliver et al. (2002); Zito et al. (2004))
PTPRF	LAR, protein tyrosine phosphatase, receptor type, F	Modulates cytoskeleton reorganization	Debant et al. (1996)
ARHGEF2	GEF-H1, rho/rac guanine nucleotide exchange factor (GEF) 2	Participates in actin filament formation and organization	(Glaven et al. (1999); Callow et al. (2005); Meiri et al. (2012))

Table 3
Clinical and demographic data for subjects included in the qRT-PCR analyses (McLean 75 cohort).

Brain	Age	Sex	PMI	Cause of death	Medications
Control					
1	81	Female	17.5	Colorectal carcinoma	Amitriptyline
2	78	Female	23	Myocardial infarction	None
3	80	Female	24	Cardiac arrest	Mirtazapine
4	48	Male	24	Myocardial infarction	None
5	50	Male	20.5	Myocardial infarction	None
6	57	Male	18	Cardiac arrest	None
7	49	Male	27	Myocardial infarction	None
8	56	Male	24.5	Cardiac arrest	None
9	65	Male	21	Cardiac arrest	None
10	41	Male	30.5	Cardiopulmonary arrest	None
11	55	Male	22	Cardiac arrest	None
12	52	Male	21.5	Cardiac arrest	None
13	46	Male	27	Atherosclerotic heart disease	None
14	55	Male	22	Myocardial infarction	None
15	49	Male	27	Cardiac arrest	None
16	36	Female	18	Cardiac arrest	Lorazepam
17	43	Male	15	Myocardial infarction	None
Schizophrenia					
18	73	Female	29	Cancer	None
19	68	Female	28	Cardiac arrest	Olanzapine, quetiapine, venlafaxine
20	46	Male	29	Cancer	Haloperidol
21	48	Male	18	Multiple myeloma	Perphenazine, quetiapine, sertraline, lorazepam
22	56	Male	22	Head trauma	Olanzapine, ziprasidone
23	58	Male	25.5	Atherosclerotic heart disease	Risperidone
24	49	Male	24.5	Acute respiratory distress	Haloperidol, triheyphenidyl
25	58	Male	26.5	Metastatic lung cancer	Haloperidol, benztropine
26	56	Female	19	Lung cancer	Olanzapine, trifluoperazine, valproic acid, lorazepam
27	62	Male	25.5	Sepsis	Olanzapine, valproic acid
28	68	Male	21.5	Cardiac arrest	Valproic acid, quetiapine, aripiprazole
29	56	Male	24.5	Asphyxia	Clozapine, valproic acid, quetiapine, haloperidol
30	55	Male	38.5	Heart failure	Risperidone, valproic acid, topiramate, benztropine, haloperidol, lorazepam
31	66	Male	16.5	Pneumonia	Clozapine, risperidone, valproic acid, lorazepam
32	60	Female	17	Cardiopulmonary arrest	Haloperidol, quetiapine, lithium
33	41	Male	33.5	Hypertensive heart disease	Chlorpromazine, lithium, benztropine, fluphenazine
34	48	Male	32.5	Suicide	Risperidone, lithium, amitriptyline
Bipolar disorder					
35	86	Female	14	Chronic obstructive pulmonary disease	Olanzapine, lorazepam, trazodone
36	77	Female	33.5	Pneumonia	Olanzapine, lorazepam, mirtazapine, gabapentin
37	78	Female	21.5	Cardiac arrest	Olanzapine, lorazepam, risperidone, gabapentin
38	51	Female	30	Suicide	Risperidone, ziprasidone, venlafaxine, duloxetine, lorazepam
39	23	Female	24	Suicide	Risperidone
40	72	Female	21.5	Metastatic breast cancer	Valproic acid, venlafaxine
41	47	Female	16.5	Major systems failure	Perphenazine, topiramate, valproic acid, clonazepam
42	80	Female	29.5	Hypernatremia	Risperidone, valproic acid, citalopram
43	66	Female	25	Suicide	Quetiapine, valproic acid, escitalopram, clonazepam, mirtazapine
44	70	Male	17.5	Renal failure	Lithium
45	76	Female	20	Aspiration pneumonia	Olanzapine, lithium
46	66	Female	25	Suicide	Quetiapine, valproic acid, escitalopram, clonazepam, mirtazapine
47	29	Male	20	Suicide	Risperidone, lithium, benztropine, diazepam
48	52	Female	17	Liver failure	Olanzapine, lithium, valproic acid
49	63	Male	27	Respiratory failure	Fluphenazine, quetiapine, lithium, valproic acid

Table 4
Summary of clinical and demographic data for subjects included in the qRT-PCR analyses (McLean 75 cohort).

	Schizophrenia	Bipolar disorder	Control	*p*-Value
Sex (M/F)	13/4	3/12	13/4	
Age	56.9 ± 8.7	63.1 ± 18.9	55.4 ± 13.4	*n.s.*
PMI (hours)	25.2 ± 6.1	21.9 ± 6.3	22.5 ± 4.1	*n.s.*
Storage time (months)	105 ± 32.2	93.1 ± 28.1	87.5 ± 19.4	*n.s.*
RIN	6.5 ± 1.1	6.9 ± 0.7	6.9 ± 0.8	*n.s.*
Suicide (Y/N)	1/16	4/11	0/17	
History of alcohol abuse or dependence (Y/N)	6/11	4/11	2/15	
History of cannabis use (Y/N)	6/11	2/13	2/15	
History of other substance abuse or dependence (Y/N)	0/17	2/13	2/15	
Antipsychotic medication (Y/N)	16/1	13/2	0/17	
Valproic acid (Y/N)	6/11	6/9	0/17	
Lithium (Y/N)	3/12	6/9	0/17	

signaling pathways. To determine if signaling pathways linking NMDA receptors and actin cytoskeleton regulatory molecules in spines have additional alterations in SZ and BD, relevant molecules having differential mRNA expression (absolute fold change > 1.2 and $p < 0.05$) in SZ and BD subjects were added. These data were previously obtained using microarrays and DLPFC tissue from the McLean 66 cohort described above.

2.4. Antipsychotic administration in rats

To assess for the potential effects of antipsychotic medication treatment, 24 adult, male Sprague Dawley rats received haloperidol 1 mg/kg/day, clozapine 25 mg/kg/day, or sterile saline (n = 8 per group) i.p. for 28 days (Merchant et al., 1994; Linden et al., 2000). They were then euthanized 24 h after the last injection. The frontal cortex (~100 mg) was dissected out and processed for qRT-PCR. The geomean of *Rpl13* and *Ppia* mRNA expression levels were used for normalization (Elfving et al., 2008).

2.5. Statistical analyses

Analysis of subject data by one-way ANOVA revealed no differences for mean age, PMI, storage time, or RIN across groups ($p > 0.05$). Relative mRNA expression was analyzed across groups using an ANCOVA. To ensure that an optimal statistical model was utilized for each gene, each of the following factors were systematically assessed alone and in combination with other factors using ANCOVA models with diagnosis and RIN included in each model. These included: age, sex, PMI, suicide, alcohol abuse or dependence, substance abuse or dependence, cannabis use, antipsychotic medication treatment in the last year of life, and treatment with lithium or valproic acid at the time of death. An optimized model was selected for each gene using the corrected Akaike's Information Criterion (AIC_C) (Akaike, 1973; Hurvich and Tsai, 1989) to identify the simplest, best-fitting model in each case. The AIC_C, which is the AIC corrected for small samples, resolves the "bias-variance tradeoff" in model selection by determining which covariates to include (to remove bias) and which to exclude (to minimize variance). To control for potential experiment-wise errors, a test-wise false-positive error rate was set at 0.05. For any gene having a significant ANCOVA effect for diagnosis ($p < 0.05$), *post-hoc* pairwise comparisons were conducted using Dunnett's method to control for multiple comparisons.

Two subjects, one SZ and one BD, had RINs < 3, thus were excluded from the qRT-PCR analyses. In addition, a brain from one SZ subject had evidence of Alzheimer's disease, thus was excluded. Moreover, several SZ (n = 3), BD (n = 2), and control (n = 1) subjects had incomplete substance abuse histories in their medical records and were excluded. Tables 3 and 4 summarize the clinical and demographic data of the SZ (n = 17), BD (n = 15) and control (n = 17) subjects included in the qRT-PCR analyses.

The relationship between relative mRNA expression of each gene and basilar dendrite parameters for pyramidal cells in the deep half of layer III in the DLPFC, obtained previously (Konopaske et al., 2014), was assessed for SZ (n = 13), BD (n = 11), and control (n = 16) subjects. A Pearson correlation coefficient was calculated for the relative mRNA expression of each gene and the number of spines per dendrite, spine density, and dendrite length.

The relationship between relative gene expression and clinical variables was assessed in SZ and bipolar groups. The following clinical variables were analyzed: suicide (yes/no), antipsychotic medication treatment in the last year of life (yes/no), lithium treatment at the time of death (yes/no) and valproic acid treatment at the time of death (yes/no). Clinical variables were analyzed with *t*-tests assuming unequal variances and *p*-values were corrected using the false discovery rate to control for multiple tests. Statistical analyses were conducted using STATA (v. 12, College Station, TX), and false discovery rate calculations were conducted using QVALUE (v. 1) (Storey, 2002).

3. Results

3.1. qRT-PCR analyses

The ANCOVA model utilized for each gene is given in Table 5. The relative expression of *MARCKS* mRNA was increased significantly in both SZ (fold change: 1.6, $p = 0.01$) and BD (fold change: 1.4, $p = 0.02$) subjects. *PPP1R9A* mRNA expression was increased significantly in BD (fold change: 4.5, $p < 0.0005$) subjects and non-significantly increased in SZ (fold change: 2.2, $p = 0.25$) subjects. *IGF1R* mRNA expression was non-significantly increased in both SZ (fold change: 1.7, $p = 0.18$) and BD (fold change: 1.6, $p = 0.2$). The relative expression of *PTPRF* and *ARHGEF2* mRNA did not differ significantly between groups ($p > 0.05$, see Fig. 1).

3.2. Effects of clinical variables

There was no difference in the relative mRNA expression of *IGF1R*, *MARCKS*, *PPP1R9A*, *PTPRF*, or *ARHGEF2* between SZ and BD subjects who did and did not commit suicide. In addition, there were no differences in relative mRNA expression of any gene when comparing SZ and BD subjects who were and were not taking antipsychotic medications in the last year of life. Likewise, treatment with either lithium or valproic acid had no effect on the relative mRNA expression of any gene.

3.3. Correlation with dendritic parameters

Relative mRNA expression was correlated with previously obtained basilar dendrite parameters of pyramidal cells localized to the deep half of layer III in the DLPFC (Konopaske et al., 2014). The relative expression of *IGF1R* mRNA had a significant negative correlation with dendrite length ($r = -0.35$, $p = 0.03$), and a moderate, negative correlation with the number of spines per dendrite which trended toward significance ($r = -0.3$, $p = 0.07$). The relative expression of *MARCKS* and *PPP1R9A* mRNA had moderate, negative correlations with dendrite length, which trended toward significance ($r = -0.29$, $p = 0.07$ and $r = -0.29$, $p = 0.08$, respectively). The relative expressions of *IGF1R*, *MARCKS*, and *PPP1R9A* mRNA did not correlate with spine density. Moreover, there was no correlation between *MARCKS* and *PPP1R9A* mRNA expression and the number of spines per dendrite. Lastly, the relative mRNA expression of *PTPRF* and *ARHGEF2* did not correlate with any dendritic parameter.

3.4. Functional pathway analyses

Functional pathway analyses revealed that *MARCKS* and *PPP1R9A* interact with NMDA receptor signaling pathways, which regulate the actin cytoskeleton and spines (Figs. 2, 3 and 4). Further analyses in SZ and BP subjects revealed altered mRNA expression of several genes which interact with NMDA signaling pathways. In SZ subjects, the relative mRNA expression of *MARCKS*, *OPHN1*, *PARD3* and *ARHGEF6* was increased (Fig. 3). In BP subjects, the expression of *MARCKS*,

Table 5
Summary of relative mRNA expression ANCOVA models.

Gene	Main effect	Covariates (in addition to RIN)
IGF1R	Diagnosis	History of alcohol abuse or dependence, age, valproic acid
MARCKS	Diagnosis	PMI
PPP1R9A	Diagnosis	Suicide
PTPRF	Diagnosis	Valproic acid
ARHGEF2	Diagnosis	PMI, valproic acid

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

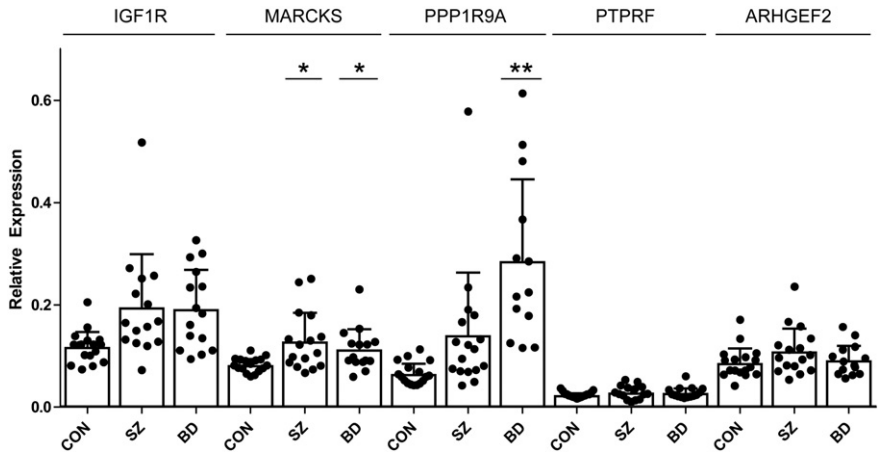

Fig. 1. Graph depicting the relative mRNA expression levels of *IGF1R*, *MARCKS*, *PPP1R9A*, *PTPRF*, and *ARHGEF2* in the DLPFC of schizophrenia (SZ), bipolar disorder (BD), and control subjects (CON). Error bars represent S.D., ** = $p < 0.01$, * = $p < 0.05$.

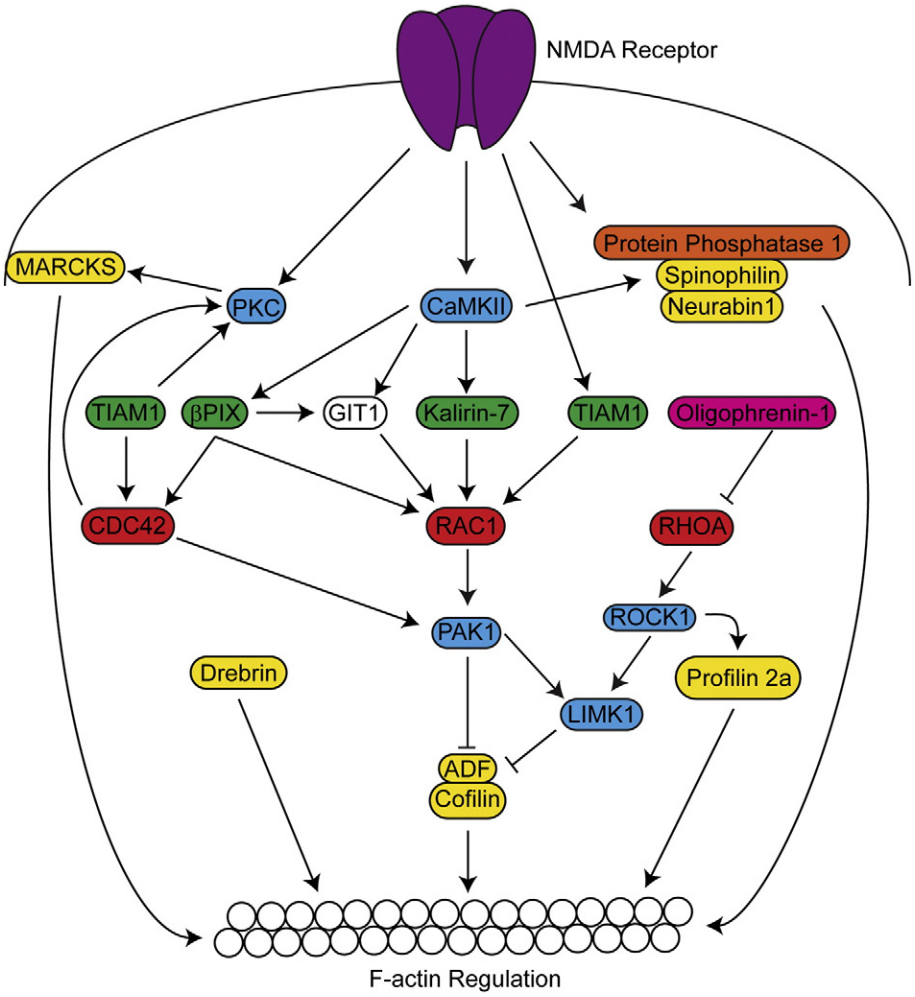

Fig. 2. Schematic depicting the regulation of the actin cytoskeleton by NMDA receptor signaling pathways. Key effector molecules are the RhoGTPases (red) which interact with actin-binding proteins (yellow) via effector molecules. Actin-binding proteins play a key role in regulating the formation, structure and function of actin filaments. NMDA receptor stimulation results in the activation of Ca^{2+}/calmodulin-dependent protein kinase CamKII which phosphorylates and activates downstream targets including the RhoGEFs (green). RhoGEFs, along with RhoGAPs (magenta), modulate the activity of the RhoGTPases. NMDA receptor activation also modulates the activity of the protein phosphatase 1/spinophilin/neurabin 1 (*PPP1R9A*) complex which regulates the actin cytoskeleton. Protein kinase C (PKC) is activated by NMDA stimulation and in turn, activates *MARCKS* which also regulates the actin cytoskeleton. In this figure, actin-binding proteins are yellow, RhoGTPases are red, kinases are blue, phosphatases are orange, RhoGEFs are green and RhoGAPs are magenta.

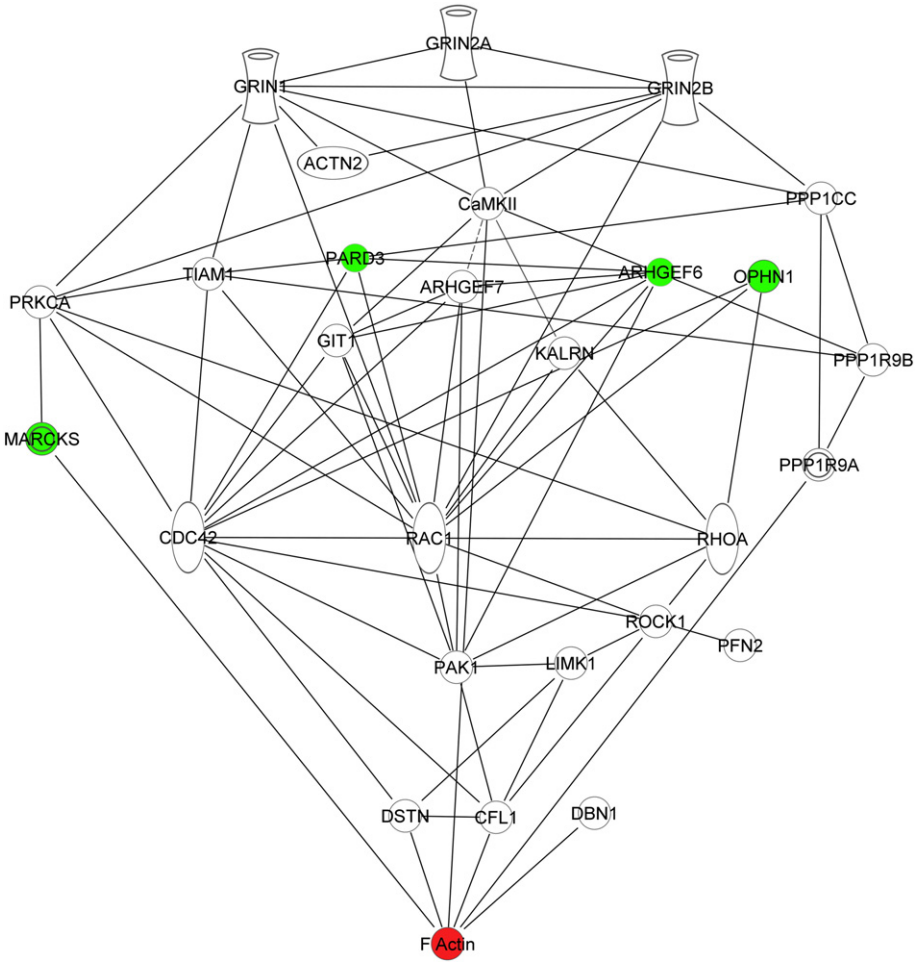

Fig. 3. Diagram depicting altered mRNA expression of molecules involved in the regulation of the actin cytoskeleton by NMDA signaling pathways in schizophrenia. Green represents increased mRNA expression, red represents decreased expression and no color represents no significant expression change. The diagram was generated using Ingenuity Pathway Analysis using data from the qRT-PCR study (double circles, McLean 75 cohort) and microarray data obtained from the DLPFC of schizophrenia subjects in the McLean 66 cohort. Both *PARD3* (par-3 family cell polarity regulator) and *ARHGEF6* (Rac/Cdc42 guanine nucleotide exchange factor (GEF) 6) regulate dendritic spine formation (Node-Langlois et al., 2006; Zhang and Macara, 2006).

PPP1R9A, *OPHN1*, and *CDC42 BPA* was increased (Fig. 4). However, the expression of *DLGAP2*, *NEFL*, and *PTK2B* was decreased. A comparison of the interaction networks for SZ and BD revealed two genes with an altered expression in both disorders, *MARCKS* and *OPHN1*.

3.5. Antipsychotic administration in rats

The relative expression of *Marcks* and *Ppp1r9a* mRNA were not changed significantly in the frontal cortex of rats chronically administered haloperidol or clozapine ($p > 0.05$, see Fig. 5).

4. Discussion

The relative expression of *MARCKS* mRNA was increased significantly in the DLPFC from both SZ and BD subjects. *PPP1R9A* mRNA expression was increased significantly in BD. The relative expression of *IGF1R* did not differ significantly among groups. However, it had a significant negative correlation with dendrite length. Although *MARCKS* and *PPP1R9A* mRNA expression did not correlate with spine loss, functional pathway analyses reveal that *MARCKS* and *PPP1R9A* interact with NMDA receptor signaling pathways, which regulate the actin cytoskeleton. In addition, several members of the NMDA signaling pathways had altered mRNA expression in the DLPFC from SZ and BD subjects, suggesting that altered regulation of the actin cytoskeleton by NMDA signaling might contribute to spine loss in both disorders.

MARCKS is expressed throughout the brain and is localized to axons, dendrites, spines and glial processes (Ouimet et al., 1990). *MARCKS* regulates the organization of actin filaments. In addition, *MARCKS* anchors actin filaments to the plasma membrane (Hartwig et al., 1992; Calabrese and Halpain, 2005; Li et al., 2008; Yamaguchi et al., 2009). The current study is consistent with a prior postmortem study finding increased MARCKS mRNA in the DLPFC (Hakak et al., 2001). Interestingly, a second study found decreased MARCKS protein in the DLPFC (Pinner et al., 2014). A knockdown of MARCKS results in spine loss (Calabrese and Halpain, 2005) therefore, decreased *MARCKS* protein expression might correlate with spine loss. In addition, the increased *MARCKS* mRNA expression observed in SZ and BD might be a compensation for decreased protein expression.

OPHN1 (oligophrenin 1) is a RhoGAP associated with X-linked mental retardation and other neurological abnormalities (Billuart et al., 1998; Bergmann et al., 2003). *OPHN1* is expressed throughout the brain, including dendrites and spines (Govek et al., 2004). *Ophn1* knockout mice exhibit phenotypes reminiscent of SZ, especially impaired spatial working memory, enlarged ventricles, and spine pathology (Khelfaoui et al., 2007, 2009).

PPP1R9A plays an important role in synaptic structure and function, neurite formation, filopodia formation, and spine motility (Nakanishi

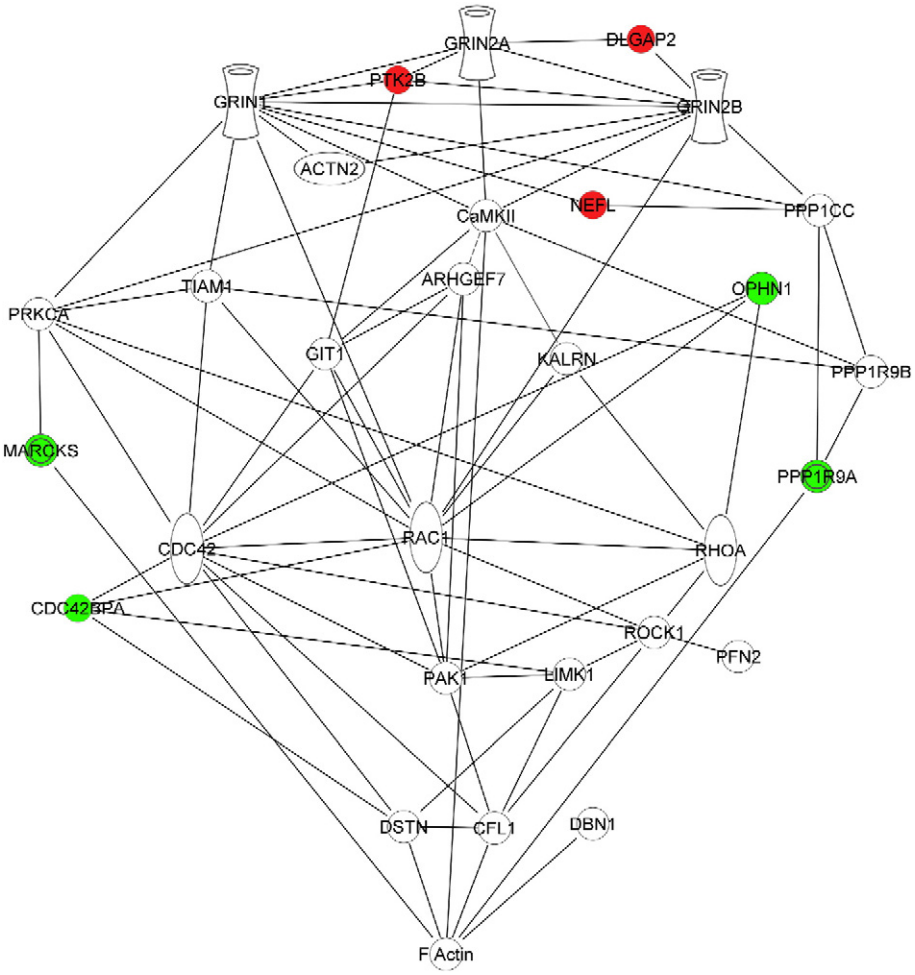

Fig. 4. Diagram depicting molecules involved in actin cytoskeletal regulation mediated by NMDA signaling pathways and having altered mRNA expression in bipolar disorder. Green represents increased mRNA expression, red represents decreased expression and no color represents no significant expression change. The diagram was generated using Ingenuity Pathway Analysis using data from the qRT-PCR study (double circles, McLean 75 cohort) and microarray data obtained from the DLPFC of bipolar disorder subjects in the McLean 66 cohort. Interestingly, mRNA expression levels of *CDC42* and *KALRN* were unchanged in contrast to a previous report (Hill et al., 2006). However, *CDC42BPA* (CDC42 binding protein kinase alpha (DMPK-like)) mRNA expression was increased. *CDC42BPA* is a *CDC42* effector protein involved in the reorganization of the actin cytoskeleton (Leung et al., 1998). *DLGAP2* (Discs, large (*Drosophila*) homolog-associated protein 2) is a post-synaptic scaffolding protein involved in the regulation of NMDA receptors and dendritic spines (Jiang-Xie et al., 2014). *NEFL* (neurofilament, light polypeptide) is a post-synaptic structural protein which regulates the surface expression and function of NMDA receptors (Ehlers et al., 1998; Ratnam and Teichberg, 2005). *PTK2B* (protein tyrosine kinase 2 beta) is involved in the regulation of NMDA receptor activity (Heidinger et al., 2002).

et al., 1997; Zito et al., 2004; Sieburth et al., 2005), and as a result we hypothesized that alterations in *PPP1R9A* mRNA expression might correlate with spine loss. As it turns out, *PPP1R9A* did not correlate with spine loss. However, *PPP1R9A* does play a role in dendritic spine morphology which could be altered in SZ and BP (Terry-Lorenzo et al., 2005; Penzes et al., 2011).

This study has some important limitations. The first involves the lack of correlation between spine loss and mRNA expression. Dendritic spine measurements were conducted in deep layer III of the DLPFC whereas the mRNA expression assessments were conducted across all layers of the grey matter and a small portion of the white matter. Differences in localization might account for the lack of correlation. The second limitation involves the relatively low RINs in some of the tissue and the necessity to use one normalizer gene due to the variability of ACTB among BP subjects. Both factors might have increased the variance and masked potential findings (Vandesompele et al., 2002; Koppelkamm et al., 2011).

Antipsychotic medication and mood stabilizer treatment can confound postmortem studies of SZ and BD. We addressed the potential confound of antipsychotics by assessing rats chronically administered haloperidol or clozapine and SZ and BD subjects who were and were not taking antipsychotic medications in the last year of life. Both data suggest that the increased *MARCKS* and *PPP1R9A* mRNA expression is not the direct result of antipsychotic medication treatment. Indeed, a prior study showed no effect of a nine month haloperidol administration on *Marcks* protein levels in rats (Pinner et al., 2014). Lithium and valproic acid have been shown to reduce *Marcks* protein levels (Lenox et al., 1992; Watson et al., 1998). We assessed SZ and BD subjects who were and were not taking lithium or valproic acid at the time of death. Neither drug appeared to affect the relative mRNA expression of *MARCKS* or *PPP1R9A*. Although the effects of antipsychotics and mood stabilizers cannot be completely ruled out, it seems unlikely that the increased *MARCKS* or *PPP1R9A* mRNA levels are the result of treatment with these medications.

In conclusion, the relative expression of *MARCKS* mRNA was increased in both SZ and BD subjects. The relative mRNA expression of *PPP1R9A* was increased in BD subjects. But, *MARCKS* and *PPP1R9A* mRNA expression did not correlate with spine loss observed in both disorders. However, functional pathway analyses of *MARCKS* and *PPP1R9A* reveal that they interact with NMDA signaling pathways that regulate the actin cytoskeleton and spines. Multiple members of this interaction network appear to have an altered mRNA

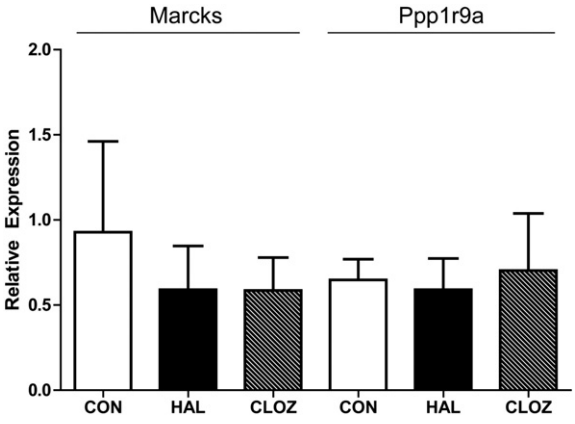

Fig. 5. Graph depicting the relative expression of *Marcks* and *Ppp1r9a* mRNA in the frontal cortex of rats chronically administered haloperidol (HAL), or clozapine (CLOZ) relative to controls (CON). No differences were detected among groups ($p > 0.05$).

expression in SZ and BD suggesting that altered actin cytoskeleton regulation by NMDA signaling pathways might contribute to the spine loss observed in both SZ and BD.

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Contributors

Glenn Konopaske participated in all phases of study design, execution, data collection, data analyses, and writing of the manuscript. Sivan Subburaju, Joseph Coyle, and Francine Benes consulted on study design and execution, contributed to data analyses, and helped edit the manuscript.

Conflict of interest

GTK, SS, and FMB have nothing to declare. JTC served as a consultant for Abbvie Laboratories and Forum Pharmaceuticals.

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References

Akaike, H., 1973. Information theory and an extension of the maximum likelihood principle. In: Petrov, B.N., Csaki, F. (Eds.), 2nd International Symposium on Information Theory. Akademia Kiado, Budapest, pp. 267–281.

Benes, F.M., Burke, R.E., Walsh, J., Berretta, S., Matzilevich, D., Minns, M., Konradi, C., 2004. Acute amygdalar activation induces an upregulation of multiple monoamine G protein coupled pathways in rat hippocampus. Mol. Psychiatry 9 (10), 932–945 (895).

Bergmann, C., Zerres, K., Senderek, J., Rudnik-Schoneborn, S., Eggermann, T., Hausler, M., Mull, M., Ramaekers, V.T., 2003. Oligophrenin 1 (OPHN1) gene mutation causes syndromic X-linked mental retardation with epilepsy, rostral ventricular enlargement and cerebellar hypoplasia. Brain 126 (Pt 7), 1537–1544.

Billuart, P., Bienvenu, T., Ronce, N., des Portes, V., Vinet, M.C., Zemni, R., Roest Crollius, H., Carrie, A., Fauchereau, F., Cherry, M., Briault, S., Hamel, B., Fryns, J.P., Beldjord, C., Kahn, A., Moraine, C., Chelly, J., 1998. Oligophrenin-1 encodes a rhoGAP protein involved in X-linked mental retardation. Nature 392 (6679), 923–926.

Calabrese, B., Halpain, S., 2005. Essential role for the PKC target MARCKS in maintaining dendritic spine morphology. Neuron 48 (1), 77–90.

Callow, M.G., Zozulya, S., Gishizky, M.L., Jallal, B., Smeal, T., 2005. PAK4 mediates morphological changes through the regulation of GEF-H1. J. Cell Sci. 118 (Pt 9), 1861–1872.

Carter, C.S., Perlstein, W., Ganguli, R., Brar, J., Mintun, M., Cohen, J.D., 1998. Functional hypofrontality and working memory dysfunction in schizophrenia. Am. J. Psychiatry 155 (9), 1285–1287.

Chang, K., Adleman, N.E., Dienes, K., Simeonova, D.I., Menon, V., Reiss, A., 2004. Anomalous prefrontal –subcortical activation in familial pediatric bipolar disorder: a functional magnetic resonance imaging investigation. Arch. Gen. Psychiatry 61 (8), 781–792.

Cheng, C.M., Mervis, R.F., Niu, S.L., Salem Jr., N., Witters, L.A., Tseng, V., Reinhardt, R., Bondy, C.A., 2003. Insulin-like growth factor 1 is essential for normal dendritic growth. J. Neurosci. Res. 73 (1), 1–9.

Cingolani, L.A., Goda, Y., 2008. Actin in action: the interplay between the actin cytoskeleton and synaptic efficacy. Nat. Rev. Neurosci. 9 (5), 344–356.

Debant, A., Serra-Pages, C., Seipel, K., O'Brien, S., Tang, M., Park, S.H., Streuli, M., 1996. The multidomain protein Trio binds the LAR transmembrane tyrosine phosphatase, contains a protein kinase domain, and has separate rac-specific and rho-specific guanine nucleotide exchange factor domains. Proc. Natl. Acad. Sci. U. S. A. 93 (11), 5466–5471.

Dillon, C., Goda, Y., 2005. The actin cytoskeleton: integrating form and function at the synapse. Annu. Rev. Neurosci. 28, 25–55.

Ehlers, M.D., Fung, E.T., O'Brien, R.J., Huganir, R.L., 1998. Splice variant-specific interaction of the NMDA receptor subunit NR1 with neuronal intermediate filaments. J. Neurosci. Off. J. Soc. Neurosci. 18 (2), 720–730.

Elfving, B., Bonefeld, B.E., Rosenberg, R., Wegener, G., 2008. Differential expression of synaptic vesicle proteins after repeated electroconvulsive seizures in rat frontal cortex and hippocampus. Synapse 62 (9), 662–670.

Feighner, J.P., Robins, E., Guze, S.B., Woodruff Jr., R.A., Winokur, G., Munoz, R., 1972. Diagnostic criteria for use in psychiatric research. Arch. Gen. Psychiatry 26 (1), 57–63.

Fukazawa, Y., Saitoh, Y., Ozawa, F., Ohta, Y., Mizuno, K., Inokuchi, K., 2003. Hippocampal LTP is accompanied by enhanced F-actin content within the dendritic spine that is essential for late LTP maintenance in vivo. Neuron 38 (3), 447–460.

Glaven, J.A., Whitehead, I., Bagrodia, S., Kay, R., Cerione, R.A., 1999. The Dbl-related protein, Lfc, localizes to microtubules and mediates the activation of Rac signaling pathways in cells. J. Biol. Chem. 274 (4), 2279–2285.

Govek, E.E., Newey, S.E., Akerman, C.J., Cross, J.R., Van der Veken, L., Van Aelst, L., 2004. The X-linked mental retardation protein oligophrenin-1 is required for dendritic spine morphogenesis. Nat. Neurosci. 7 (4), 364–372.

Hakak, Y., Walker, J.R., Li, C., Wong, W.H., Davis, K.L., Buxbaum, J.D., Haroutunian, V., Fienberg, A.A., 2001. Genome-wide expression analysis reveals dysregulation of myelination-related genes in chronic schizophrenia. Proc. Natl. Acad. Sci. U. S. A. 98 (8), 4746–4751.

Halpain, S., Hipolito, A., Saffer, L., 1998. Regulation of F-actin stability in dendritic spines by glutamate receptors and calcineurin. J. Neurosci. 18 (23), 9835–9844.

Hartwig, J.H., Thelen, M., Rosen, A., Janmey, P.A., Nairn, A.C., Aderem, A., 1992. MARCKS is an actin filament crosslinking protein regulated by protein kinase C and calcium –calmodulin. Nature 356 (6370), 618–622.

Hassel, S., Almeida, J.R., Kerr, N., Nau, S., Ladouceur, C.D., Fissell, K., Kupfer, D.J., Phillips, M.L., 2008. Elevated striatal and decreased dorsolateral prefrontal cortical activity in response to emotional stimuli in euthymic bipolar disorder: no associations with psychotropic medication load. Bipolar Disord. 10 (8), 916–927.

Heidinger, V., Manzerra, P., Wang, X.Q., Strasser, U., Yu, S.P., Choi, D.W., Behrens, M.M., 2002. Metabotropic glutamate receptor 1-induced upregulation of NMDA receptor current: mediation through the Pyk2/Src-family kinase pathway in cortical neurons. J. Neurosci. Off. J. Soc. Neurosci. 22 (13), 5452–5461.

Hill, J.J., Hashimoto, T., Lewis, D.A., 2006. Molecular mechanisms contributing to dendritic spine alterations in the prefrontal cortex of subjects with schizophrenia. Mol. Psychiatry 11 (6), 557–566.

Hurvich, C.M., Tsai, C.L., 1989. Regression and time series model selection in small samples. Biometrika 76 (2), 297–307.

Jiang-Xie, L.F., Liao, H.M., Chen, C.H., Chen, Y.T., Ho, S.Y., Lu, D.H., Lee, L.J., Liou, H.H., Fu, W.M., Gau, S.S., 2014. Autism-associated gene Dlgap2 mutant mice demonstrate exacerbated aggressive behaviors and orbitofrontal cortex deficits. Mol. Autism 5 (32), 1–13.

Khelfaoui, M., Denis, C., van Galen, E., de Bock, F., Schmitt, A., Houbron, C., Morice, E., Giros, B., Ramakers, G., Fagni, L., Chelly, J., Nosten-Bertrand, M., Billuart, P., 2007. Loss of X-linked mental retardation gene oligophrenin1 in mice impairs spatial memory and leads to ventricular enlargement and dendritic spine immaturity. J. Neurosci. Off. J. Soc. Neurosci. 27 (35), 9439–9450.

Khelfaoui, M., Pavlowsky, A., Powell, A.D., Valnegri, P., Cheong, K.W., Blandin, Y., Passafaro, M., Jefferys, J.G., Chelly, J., Billuart, P., 2009. Inhibition of RhoA pathway rescues the endocytosis defects in Oligophrenin1 mouse model of mental retardation. Hum. Mol. Genet. 18 (14), 2575–2583.

Kim, B., Feldman, E.L., 1998. Differential regulation of focal adhesion kinase and mitogen-activated protein kinase tyrosine phosphorylation during insulin-like growth factor-I-mediated cytoskeletal reorganization. J. Neurochem. 71 (3), 1333–1336.

Konopaske, G.T., Lange, N., Coyle, J.T., Benes, F.M., 2014. Prefrontal cortical dendritic spine pathology in schizophrenia and bipolar disorder. JAMA Psychiatry 71 (12), 1321–1331.

Konradi, C., Eaton, M., MacDonald, M.L., Walsh, J., Benes, F.M., Heckers, S., 2004. Molecular evidence for mitochondrial dysfunction in bipolar disorder. Arch. Gen. Psychiatry 61 (3), 300–308.

Koppelkamm, A., Vennemann, B., Lutz-Bonengel, S., Fracasso, T., Vennemann, M., 2011. RNA integrity in post-mortem samples: influencing parameters and implications on RT-qPCR assays. Int. J. Legal Med. 125 (4), 573–580.

Lawrence, N.S., Williams, A.M., Surguladze, S., Giampietro, V., Brammer, M.J., Andrew, C., Frangou, S., Ecker, C., Phillips, M.L., 2004. Subcortical and ventral prefrontal cortical neural responses to facial expressions distinguish patients with bipolar disorder and major depression. Biol. Psychiatry 55 (6), 578–587.

Lenox, R.H., Watson, D.G., Patel, J., Ellis, J., 1992. Chronic lithium administration alters a prominent PKC substrate in rat hippocampus. Brain Res. 570 (1–2), 333–340.

Leung, T., Chen, X.Q., Tan, I., Manser, E., Lim, L., 1998. Myotonic dystrophy kinase-related Cdc42-binding kinase acts as a Cdc42 effector in promoting cytoskeletal reorganization. Mol. Cell. Biol. 18 (1), 130–140.

Li, C., Wong, W.H., 2001. Model-based analysis of oligonucleotide arrays: expression index computation and outlier detection. Proc. Natl. Acad. Sci. U. S. A. 98 (1), 31–36.

Li, H., Chen, G., Zhou, B., Duan, S., 2008. Actin filament assembly by myristoylated alanine-rich C kinase substrate-phosphatidylinositol-4,5-diphosphate signaling is critical for dendrite branching. Mol. Biol. Cell 19 (11), 4804–4813.
Linden, A.M., Vaisanen, J., Lakso, M., Nawa, H., Wong, G., Castren, E., 2000. Expression of neurotrophins BDNF and NT-3, and their receptors in rat brain after administration of antipsychotic and psychotrophic agents. J. Mol. Neurosci. 14 (1–2), 27–37.
Maletic-Savatic, M., Malinow, R., Svoboda, K., 1999. Rapid dendritic morphogenesis in CA1 hippocampal dendrites induced by synaptic activity. Science 283 (5409), 1923–1927.
Manoach, D.S., Gollub, R.L., Benson, E.S., Searl, M.M., Goff, D.C., Halpern, E., Saper, C.B., Rauch, S.L., 2000. Schizophrenic subjects show aberrant fMRI activation of dorsolateral prefrontal cortex and basal ganglia during working memory performance. Biol. Psychiatry 48 (2), 99–109.
Meiri, D., Marshall, C.B., Greeve, M.A., Kim, B., Balan, M., Suarez, F., Bakal, C., Wu, C., Larose, J., Fine, N., Ikura, M., Rottapel, R., 2012. Mechanistic insight into the microtubule and actin cytoskeleton coupling through dynein-dependent RhoGEF inhibition. Mol. Cell 45 (5), 642–655.
Merchant, K.M., Dobie, D.J., Filloux, F.M., Totzke, M., Aravagiri, M., Dorsa, D.M., 1994. Effects of chronic haloperidol and clozapine treatment on neurotensin and c-fos mRNA in rat neostriatal subregions. J. Pharmacol. Exp. Ther. 271 (1), 460–471.
Nakanishi, H., Obaishi, H., Satoh, A., Wada, M., Mandai, K., Satoh, K., Nishioka, H., Matsuura, Y., Mizoguchi, A., Takai, Y., 1997. Neurabin: a novel neural tissue-specific actin filament-binding protein involved in neurite formation. J. Cell Biol. 139 (4), 951–961.
Node-Langlois, R., Muller, D., Boda, B., 2006. Sequential implication of the mental retardation proteins ARHGEF6 and PAK3 in spine morphogenesis. J. Cell Sci. 119 (Pt 23), 4986–4993.
Okamoto, K., Nagai, T., Miyawaki, A., Hayashi, Y., 2004. Rapid and persistent modulation of actin dynamics regulates postsynaptic reorganization underlying bidirectional plasticity. Nat. Neurosci. 7 (10), 1104–1112.
Oliver, C.J., Terry-Lorenzo, R.T., Elliott, E., Bloomer, W.A., Li, S., Brautigan, D.L., Colbran, R.J., Shenolikar, S., 2002. Targeting protein phosphatase 1 (PP1) to the actin cytoskeleton: the neurabin I/PP1 complex regulates cell morphology. Mol. Cell. Biol. 22 (13), 4690–4701.
Ouimet, C.C., Wang, J.K., Walaas, S.I., Albert, K.A., Greengard, P., 1990. Localization of the MARCKS (87 kDa) protein, a major specific substrate for protein kinase C, in rat brain. J. Neurosci. 10 (5), 1683–1698.
Penzes, P., Cahill, M.E., Jones, K.A., VanLeeuwen, J.E., Woolfrey, K.M., 2011. Dendritic spine pathology in neuropsychiatric disorders. Nat. Neurosci. 14 (3), 285–293.
Perlstein, W.M., Carter, C.S., Noll, D.C., Cohen, J.D., 2001. Relation of prefrontal cortex dysfunction to working memory and symptoms in schizophrenia. Am. J. Psychiatry 158 (7), 1105–1113.
Pinner, A.L., Haroutunian, V., Meador-Woodruff, J.H., 2014. Alterations of the myristoylated, alanine-rich C kinase substrate (MARCKS) in prefrontal cortex in schizophrenia. Schizophr. Res. 154 (1–3), 36–41.
Potkin, S.G., Turner, J.A., Brown, G.G., McCarthy, G., Greve, D.N., Glover, G.H., Manoach, D.S., Belger, A., Diaz, M., Wible, C.G., Ford, J.M., Mathalon, D.H., Gollub, R., Lauriello, J., O'Leary, D., van Erp, T.G., Toga, A.W., Preda, A., Lim, K.O., 2009. Working memory and DLPFC inefficiency in schizophrenia: the FBIRN study. Schizophr. Bull. 35 (1), 19–31.
Ratnam, J., Teichberg, V.I., 2005. Neurofilament-light increases the cell surface expression of the N-methyl-D-aspartate receptor and prevents its ubiquitination. J. Neurochem. 92 (4), 878–885.
Ryan, X.P., Alldritt, J., Svenningsson, P., Allen, P.B., Wu, G.Y., Nairn, A.C., Greengard, P., 2005. The Rho-specific GEF Lfc interacts with neurabin and spinophilin to regulate dendritic spine morphology. Neuron 47 (1), 85–100.
Sekino, Y., Kojima, N., Shirao, T., 2007. Role of actin cytoskeleton in dendritic spine morphogenesis. Neurochem. Int. 51 (2–4), 92–104.
Shirao, T., Gonzalez-Billault, C., 2013. Actin filaments and microtubules in dendritic spines. J. Neurochem. 126 (2), 155–164.
Sieburth, D., Ch'ng, Q., Dybbs, M., Tavazoie, M., Kennedy, S., Wang, D., Dupuy, D., Rual, J.F., Hill, D.E., Vidal, M., Ruvkun, G., Kaplan, J.M., 2005. Systematic analysis of genes required for synapse structure and function. Nature 436 (7050), 510–517.
Storey, J.D., 2002. A direct approach to false discovery rates. J. R. Stat. Soc. Ser. B 64, 479–498.
Tada, T., Sheng, M., 2006. Molecular mechanisms of dendritic spine morphogenesis. Curr. Opin. Neurobiol. 16 (1), 95–101.
Terry-Lorenzo, R.T., Roadcap, D.W., Otsuka, T., Blanpied, T.A., Zamorano, P.L., Garner, C.C., Shenolikar, S., Ehlers, M.D., 2005. Neurabin/protein phosphatase-1 complex regulates dendritic spine morphogenesis and maturation. Mol. Biol. Cell 16 (5), 2349–2362.
Vandesompele, J., De Preter, K., Pattyn, F., Poppe, B., Van Roy, N., De Paepe, A., Speleman, F., 2002. Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. Genome Biol. 3 (7) (RESEARCH0034).
Watson, D.G., Watterson, J.M., Lenox, R.H., 1998. Sodium valproate down-regulates the myristoylated alanine-rich C kinase substrate (MARCKS) in immortalized hippocampal cells: a property of protein kinase C-mediated mood stabilizers. J. Pharmacol. Exp. Ther. 285 (1), 307–316.
Yamaguchi, H., Shiraishi, M., Fukami, K., Tanabe, A., Ikeda-Matsuo, Y., Naito, Y., Sasaki, Y., 2009. MARCKS regulates lamellipodia formation induced by IGF-I via association with PIP2 and beta-actin at membrane microdomains. J. Cell. Physiol. 220 (3), 748–755.
Yurgelun-Todd, D.A., Gruber, S.A., Kanayama, G., Killgore, W.D., Baird, A.A., Young, A.D., 2000. FMRI during affect discrimination in bipolar affective disorder. Bipolar Disord. 2 (3 Pt 2), 237–248.
Zhang, H., Macara, I.G., 2006. The polarity protein PAR-3 and TIAM1 cooperate in dendritic spine morphogenesis. Nat. Cell Biol. 8 (3), 227–237.
Zito, K., Knott, G., Shepherd, G.M., Shenolikar, S., Svoboda, K., 2004. Induction of spine growth and synapse formation by regulation of the spine actin cytoskeleton. Neuron 44 (2), 321–334.