HHS Public Access

Author manuscript

J Psychiatr Res. Author manuscript; available in PMC 2017 November 01.

Published in final edited form as:

J Psychiatr Res. 2016 November ; 82: 58–67. doi:10.1016/j.jpsychires.2016.07.012.

The microRNA network is altered in anterior cingulate cortex of patients with unipolar and bipolar depression

Joshua A Azevedo[a,b], **Bradley S Carter**[a,b,c], **Fan Meng**[a,d,j], **David L Turner**[a,b,k], **Manhong Dai**[a], **Alan F Schatzberg**[d,e], **Jack D Barchas**[d,f], **Edward G Jones**[d,g], **William E Bunney**[d,h], **Richard M Myers**[d,i], **Huda Akil**[a,b,d,j], **Stanley J Watson**[a,b,d,j], and **Robert C Thompson**[a,b,d,j]

[a]Molecular and Behavioral Neuroscience Institute, University of Michigan; 205 Zina Pitcher Pl, Ann Arbor, MI, 48109, USA

[b]Neuroscience Graduate Program, University of Michigan, 4137 Undergraduate Science Building (USB), 204 Washtenaw Avenue, Ann Arbor, MI, 48109, USA

[c]Neuroscience Program, Oberlin College, Science Center A261, 119 Woodland St., Oberlin, OH, 44074, USA

[d]Pritzker Neuropsychiatric Disorders Research Consortium

[e]Department of Psychiatry and Behavioral Sciences, Stanford University, 401 Quarry Rd, Stanford, CA, 94305, USA

[f]Department of Psychiatry, Weill Cornell Medical College, 525 East 68th Street, New York, NY 10065, USA

[g]Center for Neuroscience, University of California - Davis, 1544 Newton Court, Davis, CA, 95618, USA

[h]Psychiatry and Human Behavior, University of California – Irvine, 101 The City Dr S, Orange, CA, 92868, USA

Corresponding Author: Dr. Robert C. Thompson. ***Address:*** 5049 Biomedical Science Research Building, 109 Zina Pitcher Place, Ann Arbor, MI, 48109. ***Phone:*** 734-615-3117 ***Fax:*** 734-647-4130.

Role of Contributors

Joshua A Azevedo: study design, data analysis, manuscript drafting and final revision of manuscript.
Bradley S Carter: early-stage technical feasibility, final revision of manuscript.
Fan Meng: bioinformatics analysis, final revision of manuscript.
David L Turner: bioinformatics analysis, final revision of manuscript.
Manhong Dai: bioinformatics analysis, final revision of manuscript.
Alan F Schatzberg: overall study design and post-mortem sample procurement, final revision of manuscript.
Jack D Barchas: overall study design and post-mortem sample procurement, final revision of manuscript.
Edward G Jones: supervised and organized neuroanatomical dissection of human post-mortem tissue.
William E Bunney: supervised sample collection, overall study design and post-mortem sample procurement,
Richard M Myers: overall study design and post-mortem sample procurement, final revision of manuscript.
Huda Akil: overall study design and post-mortem sample procurement, final revision of manuscript.
Stanley J Watson: overall study design and post-mortem sample procurement, final revision of manuscript.
Robert C Thompson: study design, data analysis and final revision of manuscript.
All authors have approved the final version of this manuscript.

[i]Hudson Alpha Institute for Biotechnology, 601 Genome Way Northwest, Huntsville, AL 35806, USA

[j]Department of Psychiatry, University of Michigan, 530 Church St, Ann Arbor, MI 48109, USA

[k]Department of Biological Chemistry, University of Michigan, 5301 MSRB III, 1150 W. Medical Center Drive, Ann Arbor, MI, 48109, USA

Abstract

MicroRNAs (miRNAs) are small, non-coding RNAs acting as post-transcriptional regulators of gene expression. Though implicated in multiple CNS disorders, miRNAs have not been examined in any psychiatric disease state in anterior cingulate cortex (AnCg), a brain region centrally involved in regulating mood. We performed qPCR analyses of 29 miRNAs previously implicated in psychiatric illness (major depressive disorder (MDD), bipolar disorder (BP) and/or schizophrenia (SZ)) in AnCg of patients with MDD and BP versus controls. *miR-132, miR-133a* and *miR-212* were initially identified as differentially expressed in BP, *miR-184* in MDD and *miR-34a* in both MDD and BP (although none survived multiple correction testing and must be considered preliminary). *In silico* target prediction algorithms identified putative targets of differentially expressed miRNAs. Nuclear Co-Activator 1 (*NCOA1*), Nuclear Co-Repressor 2 (*NCOR2*) and Phosphodiesterase 4B (*PDE4B*) were selected based upon predicted targeting by *miR-34a* (with *NCOR2* and *PDE4B* both targeted by *miR-184*) and published relevance to psychiatric illness. Luciferase assays identified *PDE4B* as a target of *miR-34a* and *miR-184*, while *NCOA1* and *NCOR2* were targeted by *miR-34a* and *184*, respectively. qPCR analyses were performed to determine whether changes in miRNA levels correlated with mRNA levels of validated targets. *NCOA1* showed an inverse correlation with *miR-34a* in BP, while *NCOR2* demonstrated a positive correlation. In sum, this is the first study to demonstrate miRNA changes in AnCg in psychiatric illness and validate *miR-34a* as differentially expressed in CNS in MDD. These findings support a mechanistic role for miRNAs in the regulation of stress-responsive genes disrupted in psychiatric illness.

Introduction

Known as *melancholia* at the time of Hippocrates, ‘depression’ is a general term that encompasses a large number of mood disorders. Two of these particularly debilitating disorders—major depressive disorder (MDD, or unipolar depression) and bipolar disorder (BP; bipolar depression)—are also extremely common, with a lifetime prevalence of 16.6% and 3.9%, respectively (Kessler et al., 2005). Though a genetic component has been established (due in part to a high degree of heritability (Bierut et al., 1999, Burton et al., 2007, Lohoff, 2010, McGuffin et al., 2003, Sklar et al., 2011, Smoller and Finn, 2003)), the genomic architecture of these disorders remains poorly understood.

In recent years, however, microRNAs (miRNAs)—small, 21–23 nt RNAs that canonically act as post-transcriptional regulators of gene expression—have become an increasing focus for understanding CNS processes. Greater than 40% of all protein-coding transcripts are predicted to be regulated by miRNAs (Tan et al., 2009, Xie et al., 2005). MiRNAs are also highly enriched within the CNS, with greater than two-thirds of identified miRNAs

expressed in brain (Bak et al., 2008, Cao et al., 2006, Sempere et al., 2004). MiRNAs are also key governors of CNS processes at both the cellular level (e.g. synaptic plasticity, neuronal differentiation and neuronal migration (Cui et al., 2012, Makeyev et al., 2007, Morgado et al., 2014, Schratt et al., 2006)) and the systems level, with miRNAs linked to the regulation of HPA axis glucocorticoid negative feedback and complex behaviors such as responses to both acute and chronic stress as well as mood and anxiety (Bahi et al., 2014, Haramati et al., 2011, Honda et al., 2013, Katsuura et al., 2012, Muinos-Gimeno et al., 2011, Vreugdenhil et al., 2009).

The role of miRNAs in the regulation of stress responses is of particular interest given that chronic stress is not only a precipitant of mood and affective disorders (Breslau and Davis, 1986, Ilgen and Hutchison, 2005) but HPA axis disruption is one of the most commonly observed pathophysiologies in MDD patients, with symptomatic severity correlating with extent of hypercortisolemia (Gibbons and Mc, 1962, Vythilingam et al., 2004). Intriguingly, a number of studies have directly demonstrated dysregulation of the miRNA regulatory network in patients with a variety of mood and affective disorders, with the vast majority focusing on schizophrenia (SZ) (Beveridge et al., 2010, Beveridge et al., 2008, Kim et al., 2010, Miller et al., 2012, Moreau et al., 2011, Perkins et al., 2007, Santarelli et al., 2011, Shi et al., 2012, Smalheiser et al., 2014, Wan et al., 2015). Absent from these studies, however, has been analysis of the anterior cingulate cortex (AnCg), a brain region centrally involved in the regulation of mood, affect and cognition (Drevets et al., 2008, Ebert and Ebmeier, 1996, Mayberg et al., 1999, Posner and DiGirolamo, 1998). Alterations in AnCg function have been increasingly linked to mood disorders with AnCg activity previously demonstrated to differentiate patients with unipolar versus bipolar depression (Diler et al., 2014) and also to predict successful pharmaceutical and cognitive treatment response (Fujino et al., 2015, Mulert et al., 2007, Pizzagalli et al., 2001, Salvadore et al., 2009). Further work has also established alterations in various systems within AnCg in MDD and BP disorders, including dysregulation in the fibroblast growth factor (FGF) system and clock genes (Bunney et al., 2015, Cheng et al., 2007, Evans et al., 2004).

In the present study we assessed miRNA expression in the AnCg of both MDD and BP patients compared to controls. As miRNAs exert their regulatory effects by targeting mRNA transcripts, we employed bioinformatics approaches to identify mRNA targets of miRNAs whose expression varied due to disease and validated several mRNAs as direct targets. Finally, we examined the steady-state levels of a subset of validated mRNA targets and identified two that vary as a function of affective disease.

Materials and Methods

Postmortem brain tissue and RNA extraction

RNA samples derived from human post-mortem AnCg tissue were provided by the Pritzker Neuropsychiatric Research Consortium. The initial acquisition of tissue, microdissection of AnCg and subsequent RNA extraction that generated these samples is described in detail in (Evans et al., 2003). Briefly, brains were extracted during autopsy and sliced into coronal slabs approximately 0.75 cm thick. Slabs were then snap-frozen and stored at −80 degrees C until subsequent dissections. Anterior cingulate cortex (AnCg, corresponding to Brodmann's

Area 24) was identified and dissected from left hemisphere, with all dissections being performed with tissue slabs on dry ice. Following dissection, total RNA was extracted from each sample using TRIzol (Invitrogen).

Patient demographics and information—including gender, brain pH, post-mortem interval, medication history and agonal factor status—are listed in Supplementary Table 1. A total of 37 patients (n=8, BP; n=15, MDD; n=14, Control) were used for all miRNA and mRNA qPCR experiments. Patient diagnoses were based on criteria from the Diagnostic and Statistical Manual of Mental Disorders, 4th Edition, and were obtained from medical examiners, medical records and a family member. Patient samples were matched on two primary criteria, brain pH and agonal factor score. Given that our group has previously observed the significant impact of low brain pH on gene expression (Li et al., 2004) all patients were noted to have a brain pH ≥ 6.55 to mitigate gene expression variance. Additionally, as a prolonged agonal state (e.g. coma, multiple organ system failures, respiratory arrest, etc.) tended to be associated with lower brain pH, all patients included in this study had no agonal factors.

MicroRNA selection, reverse transcription and detection

29 MicroRNAs (Supplementary Table 2) were selected for qPCR analyses. These miRNAs were based upon several criteria including prior published association with psychiatric illness(es) at the time of miRNA selection, shared dysregulation between multiple psychiatric illnesses (e.g. SZ, BP and/or MDD), abundant expression and prior literature validating interactions with mRNAs previously implicated in mental illness (outlined in Supplementary Table 2). Total human RNA (7.5 ng) was reverse transcribed with the High Capacity RNA to cDNA Kit (Applied Biosystems Inc., Carlsbad, CA) as per manufacturer's instructions using custom pooled RT primers corresponding to miRNAs selected for analysis. Following reverse transcription, first-strand cDNA was subjected to preamplification per manufacturer's instructions (Applied Biosystems Inc., Carlsbad, CA) using custom pooled preamplification primers. The resulting preamplified material was diluted 1:4 in 0.1× TE buffer before being subjected to qPCR. qPCR was performed using custom TaqMan Low-Density Array (TLDA) cards (Applied Biosystems Inc., Carlsbad, CA). Each TLDA card accommodated 4 biological samples and measured 29 miRNAs, as well as RNU48 as a control, in technical triplicate. RNU48 showed no significant variability between patient cohorts (CTRL, MDD and/or BP). qPCR reactions were run and measured on a ViiA7 thermocycler (Applied Biosystems Inc., Carlsbad, CA) using the following conditions: 2 minutes at 50 C, 10 minutes at 95 C (1 repeat); 15 seconds at 95 C, 1 minute at 60 C (40 repeats). Following detection, miRNAs were analyzed for differential expression using the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen, 2001).

In silico target prediction and vector construction

Putative mRNA targets of dysregulated miRNAs were identified based on predicted targeting in miRanda (Betel et al., 2010) (August 2010 Release) and/or TargetScan (Lewis et al., 2005) (Release 6.2) *in silico* target prediction algorithms using default parameters. A gene ontology analysis of predicted targets of miR-34a was performed using DAVID Bioinformatics (Huang da et al., 2009a, b) using default parameters (Supplementary Table

3). Following this, candidate mRNA targets were narrowed to those associated with terms in the top-scoring functional clusters. Targets were subsequently selected for validation based upon 1) a direct association (based upon prior literature) with neuropsychiatric illness and/or 2) function in a biological process implicated in the pathophysiology of mental illness (e.g. glucocorticoid signaling, synaptic plasticity, transcriptional regulation). Target genes were amplified via end-point PCR (Supplementary Table 4) and subsequently cloned into a previously described US2 plasmid expression vector encoding firefly luciferase driven by the human UBC promoter (US2-Luc) (Yu et al., 2008). Putative miRNA binding sites were identified via the aforementioned prediction algorithms and subsequently mutagenized using the QuikChange XL kit (Agilent Technologies, Santa Clara, CA) following manufacturer's protocol. Mutagenesis primers (Supplementary Table 4) were designed using the web-based QuikChange Primer Design software (Agilent Technologies, Santa Clara, CA).

Cell culture, transfection and luciferase assays

96-well plates were coated for 15 minutes in 0.1 mg/mL poly-L-lysine (ThermoFisher Scientific, Waltham, MA) and washed with 1X PBS to aid in cell adherence. HEK293 cells were plated at 70–90% confluence in 75 μl DMEM media (Invitrogen, Carlsbad, CA) containing Pen-Strep (Life Technologies, Carlsbad, CA) and 10% FBS (ThermoFisher Scientific, Waltham, MA) per well. Cells were maintained at 37 C at 5% CO_2.

24 hours following plating HEK293 cells were transfected with 0.5 μl Lipofectamine 2000 (Invitrogen, Carlsbad, CA), 300 ng US2-Firefly Luciferase (US2-Luc) plasmid containing the 3' UTR of a gene of interest, 300 ng US2-Renilla Luciferase (US2-RL) plasmid and either 0.5 pmol miRNA mimic (MISSION MicroRNA Mimic) (Sigma-Aldrich, St. Louis, MO) (miRNA treated) or 1 μl sterile saline (vehicle treated) in 50 μl OPTI-MEM media (Life Technologies, Carlsbad, CA) per well (n=6 per treatment group). Cells were allowed to incubate for 24 hours before proceeding to luciferase assays.

Immediately prior to luciferase assays, OPTI-MEM media was aspirated and replaced with 75 μl of DMEM media per well. Protein lysates were prepared by adding 75 μl of Dual-Glo Luciferase Assay Reagent (Promega, Madison, WI) per well and transferring the resulting lysate to a 96-well microassay plate (Sigma-Aldrich, St. Louis, MO). Luciferase assays were then performed following manufacturer's instructions (Dual-Glo® Luciferase Assay System (Promega, Madison, WI)). Luminescence was measured on a FluoStarOptima (BMG Labtech, Germany) with renilla luciferase serving as a transfection efficiency control.

Messenger RNA reverse transcription and qPCR detection

The same RNA samples used for the miRNA expression analyses were used for messenger RNA studies. Briefly, RNA samples (25 ng per sample) were converted to cDNA with random hexamer priming using SuperScript II Reverse Transcriptase (Invitrogen, Carlsbad, CA) following manufacturer's instructions. The resulting cDNA was diluted 1:4 in sterile water before use in subsequent qPCR studies.

Individual TaqMan gene expression assays (Applied Biosystems Inc., Carlsbad, CA) were used to measure gene expression for *NCOA1* (Assay ID# HS00186661_m1), *NCOR2* (Assay ID# HS00196955_m1) and *PDE4B* (Assay ID# HS00963643_m1) with beta actin

(Assay ID# HS99999903_m1) serving as a reference control. Messenger RNA qPCR detections were run on a ViiA7 thermocycler (Applied Biosystems Inc., Carlsbad, CA) using the previously described thermocycler conditions. All samples were run in technical triplicate and were analyzed for differential expression using the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen, 2001).

Data Analysis

For miRNA expression values, raw P-values were generated using a two-tailed Student's T-test. Multiple correction testing was performed with the Benjamini-Hochberg False Discovery Rate (FDR; Benjamini and Hochberg, 1995) with FDR set at 15%. Luciferase assays were assayed for significance using a two-tailed Student's T-test with significance set at $p < 0.05$.

Results

Differential expression of a subset of miRNAs in AnCg of patients with MDD or BP disorder

Following qPCR detection 3 miRNAs—*miR-33a*, *miR-144* and *miR-431**—were excluded from analysis due to high variability among technical replicates and cycle threshold values >30. After exclusion, 26 miRNAs were examined for differential expression in BP and MDD cohorts versus controls. Of these, 5 miRNAs—*miR-132*, *miR-133a* and *miR-212* in the BP cohort; *miR-184* in the MDD cohort, and *miR-34a* shared between cohorts—exhibited raw p-values < 0.05 (Figure 1) (although none passed multiple correction testing, e.g. FDR > 0.15).

Intriguingly, these miRNAs were dysregulated in a unidirectional fashion: all dysregulated miRNAs were repressed compared to control patients. While it also failed to achieve significance, *miR-195*—previously linked to regulation of brain-derived neurotrophic factor (*BDNF*) (Mellios et al., 2008)—exhibited a trend towards repression in both BP and MDD cohorts (p=0.10 and p=0.09, respectively). We note that three of these miRNAs—*miR-132*, *miR-212* and *miR-34a*—have previously been shown to be dysregulated in PFC of SZ patients (Kim, Reimers, 2010, Miller, Zeier, 2012). These results suggest the shared dysregulation of several miRNAs across several neuropsychiatric conditions, with *miR-34a* serving as a consistently dysregulated miRNA in MDD, BP and SZ. Given this, along with the large number of validated miR-34a targets previously linked to multiple mood and affective disorders (Table 1), we elected to focus primarily on miR-34a for subsequent analyses.

In silico target prediction analyses of dysregulated miRNAs

Following the identification of *miR-34a* as differentially expressed, we performed a gene ontology analysis on the 655 putative *miR-34a* targets identified by the TargetScan algorithm (Lewis, Burge, 2005). Based upon the enriched terms in the two most significant GO clusters— specifically, 'Synapse' (Cluster 1) and 'Transcription Regulator Activity' (Cluster 2) (Supplementary Table 3)—we were able to identify 3 putative targets of *miR-34a* based upon our previously described selection criteria. Prior work has shown that two of these genes (*NCOA1* and *NCOR2*) modulate the transcriptional activity of the

glucocorticoid receptor (van der Laan et al., 2008), while *PDE4B*—a genetic risk factor for mental illness (Fatemi et al., 2008, Millar et al., 2005, Numata et al., 2009, Pickard et al., 2007)—regulates cAMP signaling and is enriched at the synapse (Bradshaw et al., 2008, Millar, Pickard, 2005). Further sequence analysis indicated that, in addition to putative *miR-34a* binding sites, both *NCOR2* and *PDE4B* possess putative *miR-184* binding sites, potentially indicating common regulatory targets for both miRNAs disrupted in MDD patients.

Target validations of dysregulated miRNAs

Following the identification of *NCOA1*, *NCOR2* and *PDE4B* as putative mRNA targets of dysregulated miRNAs, luciferase vectors containing their respective 3' UTRs were generated as previously described (Yu, Chung, 2008). HEK293 cells transfected with US2-Luc plasmids containing a wild-type 3' UTR from *PDE4B* (*PDE4B* WT 3' UTR) and either a miR-34a mimic or a miR-184 mimic yielded reduced luciferase values relative to control (Fig. 2a) (n=6 per treatment group). Similarly, HEK293 cells transfected with *NCOA1* or *NCOR2* WT 3' UTR yielded reduced luciferase activity when transfected with *miR-34a* or *miR-184* mimics, respectively (Fig. 2b and 2c). Though it exhibited a strong trend, treatment of HEK293 cells transfected with *NCOR2* WT 3' UTR and miR-34a did not result in a statistically significant repression of luciferase activity (Fig. 2c). The specificity of these mRNA/miRNA interactions were demonstrated when mutating predicted *miR-34a* or *miR-184* binding sites (34a-MUT or 184-MUT, respectively) was sufficient to relieve repression in the presence of miRNA mimics (Fig. 2a–c). These findings demonstrate the direct regulation of these genes of interest by miRNAs dysregulated in BP and MDD patients.

qPCR of validated miRNA targets

As one way that miRNAs can exert their regulatory influence is via the degradation of mRNA transcripts, we employed qPCR methodologies to examine steady-state levels of our validated targets of *miR-34a* and/or *miR-184* (Fig. 3). Consistent with miRNAs' canonical role as negative regulators of gene expression, we observed a significant increase in *NCOA1* mRNA levels in the BP cohort but not the MDD cohort. In contrast, we observed a significant *decrease* in *NCOR2* mRNA levels specific to the MDD cohort. Neither BP nor MDD cohorts showed significant alterations in *PDE4B* mRNA expression. Taken together, these results suggest the possibility that the reduction in *miR-34a* levels may influence steady-state levels of *NCOA1* in BP patients.

Discussion

Given their enrichment in the brain, their regulation of key CNS processes, their widespread regulation of protein-coding transcripts and their dysregulation in a number of illnesses, miRNAs are uniquely positioned to play a key role in the pathology of psychiatric illness. In this study we examined the expression of 26 miRNAs in the AnCg of MDD and BP patients versus controls. From this, we identified 5 miRNAs—3 in BP, 1 in MDD and 1 shared across both cohorts—that were differentially expressed in patients with psychiatric illness. Intriguingly, fold changes were unidirectional with all differentially expressed miRNAs

reduced in MDD or BP versus controls. Additionally, we examined a subset of putative targets for differentially expressed miRNAs, and were able to validate *NCOA1* as a target of *miR-34a*, *NCOR2* as a target of *miR-184* and *PDE4B* as a target of both *miR-34a* and *miR-184*. mRNA levels of *NCOA1* showed an inverse correlation with *miR-34a* in the BP cohort, while mRNA levels of *NCOR2* showed a positive correlation with *miR-34a* and *miR-184* in the MDD cohort (with *PDE4B* showing no expression change in either). While we note that it is possible that repression may occur at the level of translation rather than transcription (Wilczynska and Bushell, 2015), patient samples were not initially processed for protein examination; as such, we were unable to investigate this line of inquiry. While we recognize that the observed changes in miRNA expression levels are relatively modest, we feel it important to note that cooperative repression by a miRNA in combination with other miRNAs or RNA-binding proteins allows a non-linear relationship between miRNA expression and extent of mRNA target repression (Broderick et al., 2011, Jacobsen et al., 2010, Mukherji et al., 2011), potentially allowing even minor changes in miRNA levels to act as ‘switches’ rather than simply fine-tuning gene expression.

These data add to our understanding of potential mechanisms underlying psychiatric disorders. While miRNA expression has been examined across a multitude of psychiatric illnesses, encompassing several brain regions, cell types and blood (Bavamian et al., 2015, Fan et al., 2014, Kim, Reimers, 2010, Miller, Zeier, 2012, Moreau, Bruse, 2011, Sun et al., 2015, Walker et al., 2015), to our knowledge this is the first work to examine miRNAs dysregulated as a function of psychiatric illness in AnCg. Additionally, comparing prior work examining miRNA dysregulation in the DLPFC—another brain region of intense interest in the pathology of mental illness—of BP and MDD patients to our results in AnCg, we see little overlap in the specific miRNA species dysregulated as a function of anatomy (e.g. brain region). Potential explanations for this lack of overlap across studies include the usage of different cohorts of patients across multiple studies (including our own which, in turn, severely limits our ability to draw parallels between miRNA expression between brain regions in the present study) and the heterogeneity of neuropsychiatric illness. However, given prior work revealing that the complement of transcripts dysregulated in mental illness is highly dependent on brain region, the notion of region-specific patterns in miRNA disruption is consistent with current knowledge.

Of further note is that this specificity extends not only to miRNA species (e.g. *miR-34a* versus *miR-132*) but also to individual miRNA isoforms. In a prior study, *miR-133b* was differentially expressed in DLPFC of BP patients (Kim, Reimers, 2010). In contrast, while *miR-133b* levels did not change in our cohort of MDD and BP patients versus controls, *miR-133a* (encoded by a different gene than *miR-133b*) was differentially expressed in the AnCg of our cohort of BP patients (Fig. 1). As the canonical mature forms of *miR-133a* and *b* differ in only one nucleotide (a U versus a G, respectively, at the final 3’ residue), this single-nucleotide difference could represent a more subtle (but potentially significant) shift in target recognition between the two isoforms. However, as canonical miRNAs represent one of several abundant isoforms of the mature miRNA (each of which may have different nucleotides at the 5’ and 3’ ends), this hypothesis remains speculative.

While *miR-132* and *miR-212* have been previously identified as differentially expressed in the DLPFC of SZ patients (Kim, Reimers, 2010, Miller, Zeier, 2012), we have identified these miRNAs—which are co-transcribed in the same primary transcript—as differentially expressed in a cohort of BP patients. This finding is intriguing as BP and SZ may share familial and genetic risk factors (Berrettini, 2003, Kim et al., 2015, Purcell et al., 2009, Shepherd et al., 2015) including miRNAs (Kim, Reimers, 2010, Miller, Zeier, 2012, Walker, Rybka, 2015) and, given the seed sequences of *miR-132* and *miR-212* are identical, these miRNAs may share a number of targets. Additionally, as *miR-34a* expression levels are dysregulated in cohorts of both SZ and BP patients (in DLPFC and cerebellum, respectively) (Bavamian, Mellios, 2015, Kim, Reimers, 2010), our finding that *miR-34a* is significantly differentially expressed in a cohort of patients with MDD identifies *miR-34a* as the first miRNA to be differentially expressed in the CNS across 3 psychiatric illnesses—BP, MDD and SZ. Unfortunately, the lack of SZ patients in the present study precludes the possibility of identifying whether *miR-34a* is regulated across BP, MDD and SZ specifically in AnCg: an intriguing possibility given the postulated linkage between BP and SZ (Berrettini, 2003, Purcell, Wray, 2009) and several shared symptoms of BP and MDD.

Given prior evidence that suicide may be a strong factor in influencing miRNA expression (Smalheiser, Lugli, 2014) we also performed miRNA expression analyses specifically in the suicide subgroups of our MDD and BP cohorts. Analyses of miRNA expression in the suicide subgroups of MDD and BP mirrored the directionality, magnitude of fold-change and disease specificity compared to entire patient populations. For example, *miR-184* demonstrated a linear fold change of 0.84 and 0.85, while *miR-34a* exhibited a linear fold change of 0.78 and 0.76 (both in suicide- and non-suicide MDD patients, respectively). While these miRNA expression analyses using only non-suicide patients revealed trends in the directionality of fold change and disease specificity identical to both suicide-subgroup and whole-group analyses, these changes also failed to achieve statistical significance. We note, however, that the total number of patients in the non-suicide groups are extremely small (n=2, BP; n=5, MDD) and, as such, we are unable to definitively conclude whether suicide defined a subgroup of BP or MDD subjects.

miR-34a and *miR-184*'s respective regulation of *NCOA1* and *NCOR2*—which, in turn, can alter the glucocorticoid receptor's (GR) transcriptional activity (Lachize et al., 2009, van der Laan, Lachize, 2008)—is intriguing for several reasons. *NCOA1* has been identified as necessary for proper stress responses (Lachize, Apostolakis, 2009, Winnay et al., 2006) while prior literature demonstrates both *NCOA1* and *NCOR2* modulate the GR-mediated transcription of corticotropin releasing hormone (*CRH*), a psychiatric risk factor linked to MDD pathology (van der Laan, Lachize, 2008). Prior work has demonstrated the direct transcriptional repression of *miR-184* by the psychiatric risk factor methyl CpG binding protein 2 (MeCP2) (Nomura et al., 2008). Additionally, the *miR-34* family (including *miR-34a*) has previously been linked to stress (Haramati, Navon, 2011) and is strongly induced by the *TP53* gene, a key cell-cycle control gene that also regulates expression of the mood-stabilizing *Wip1* gene (Rokavec et al., 2014, Ruan et al., 2015). Chronic stress is thought to act as a precipitating environmental factor for a host of mental illnesses and induces chronic elevations in circulating glucocorticoids (e.g. hypercortisolemia). Altered HPA axis activity—particularly hypercortisolemia—is one of the most consistently observed

pathophysiologies in MDD patients (Gibbons and Mc, 1962) and is observed in both BP and SZ (Altamura et al., 1999, Daban et al., 2005, Jakovljevic et al., 1998). As glucocorticoids exert powerful transcriptional effects through the GR (with GR mRNA level significantly reduced in MDD versus control patients in a prior study (Qi et al., 2013)) and *miR-34a* exerts influence over a GR cofactor, these findings suggest a role for *miR-34a* in the transcriptional response to stress. The authors acknowledge the limitations inherent to proposing direct linkages between miRNA and mRNA function. Specifically, we note that the polygenic and heterogeneous molecular architecture of depressive illnesses make it extremely difficult to couple the impact of an individual miRNA with a specific mRNA. While our hypotheses are consistent with our present data, future studies are of the utmost importance to characterize the prospective impact(s) of miRNAs dysregulated in disease.

The shared regulation of *PDE4B* by *miR-34a* and *miR-184* is also of note given *PDE4B*'s linkage to MDD, BP, SZ and anxiety (Fatemi, King, 2008, McGirr et al., 2015, Millar, Pickard, 2005, Numata, Iga, 2009, Padmos et al., 2008, Pickard, Thomson, 2007, Yuan et al., 2011). In addition to PDE4B binding to and being regulated by the psychiatric risk factor *Disrupted in Schizophrenia 1* (*DISC1*) (Millar, Pickard, 2005), pharmacological inhibitors of *PDE4B* activity have previously been tested as atypical antidepressants (Fleischhacker et al., 1992, Zeller et al., 1984). While these drugs were never widely deployed (due primarily to negative side-effects at therapeutic dosage), the directionality suggested by this pharmacological treatment—i.e. that *increased PDE4B* is correlated with MDD—is substantiated by prior work demonstrating an increase in levels of PDE4B protein in cingulate cortex of MDD patients versus controls (Yuan, Tragon, 2011). This prior work is also consistent with our current data, in which decreases in miR-34a and *miR-184* (Fig. 1)—which can negatively regulate *PDE4B* (Fig. 2)—should lead to an increase in *PDE4B* protein levels. While *PDE4B* mRNA levels were unchanged in either BP or MDD cohorts of the present study, it is important to note that miRNAs can exert their regulatory influence through translational inhibition as well as via mRNA degradation (reviewed in (Wilczynska and Bushell, 2015)).

We note that—in addition to the work presented here—several groups have validated additional targets of *miR-34a* that are either directly dysregulated or indirectly implicated in biological processes thought to be disrupted in psychiatric disease (Table 1). Given that mental illnesses are noted for their heterogeneity of causes, symptoms and treatments, it is noteworthy that *miR-34a*—representing the only shared miRNA dysregulated in MDD, BP and SZ—targets transcripts implicated in the pathology of all three of these illnesses (Table 1). Intriguingly, *miR-34a* has been linked to acute responses to stress while another *miR-34* isoform—*miR-34c*—was differentially expressed in animal models of both acute and chronic stress (Haramati, Navon, 2011). As stress-induced HPA axis dysfunction is a common precipitating event for mood and affective disorders—and our work presented here identifies *miR-34a* as a regulator of *NCOA1*, itself previously identified as a key modulator of both stress responses and HPA axis activity (Lachize, Apostolakis, 2009, Winnay, Xu, 2006)—these findings suggest *miR-34a* as an attractive target for further investigation.

Finally, we note several additional limitations and caveats in our study. We note that none of the miRNAs we identified as differentially expressed have passed multiple correction testing

and, as such, must be regarded as preliminary. Limitations when using the Student's T-test in modest sample sizes (such as those employed here) have also been well described, including poor reliability and reproducibility (reviewed in Halsey et al, 2015). We also acknowledge that, as we employed an *in vitro* model system using HEK293 cells engineered to express both the 3' UTR of our mRNA target and our miRNA(s) of interest, future studies employing human neural cell types will be necessary to assess the biological relevance of the interactions.

In sum: due to their enrichment in the brain, regulation of key CNS processes and the fact one miRNA can regulate hundreds of mRNA targets, miRNAs are hypothesized to play a key role in mood and affective disorders. While we acknowledge several caveats and limitations, this current work sheds light on (albeit prelimnary) putative miRNA dysregulation in AnCg (a brain region central to the regulation of mood and cognition) as well as the mRNA targets of these dysregulated miRNAs. We also identify *miR-34a* in particular as a miRNA dysregulated across multiple psychiatric illnesses and across multiple cortical brain regions known to participate in affect and whose role in the molecular architecture of these disorders remains to be fully described.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by NIH Grant R21 MH083175 (RCT). J. Azevedo was supported by NIH T-32-NS076401. This work was also funded by the Pritzker Neuropsychiatric Disorders Research Consortium, which is supported by the Pritzker Neuropsychiatric Disorders Research Fund L.L.C. A shared intellectual property agreement exists between this philanthropic fund and the University of Michigan, Stanford University, the Weill Medical College of Cornell University, the University of California at Irvine, and the Hudson Alpha Institute for Biotechnology to encourage the development of appropriate findings for research and clinical applications.

Role of the Funding Sources

No sponsors of this research played a role in study design, data acquisition or interpretation, writing of the report or in the decision to submit this article for publication.

References

Abe N, Uchida S, Otsuki K, Hobara T, Yamagata H, Higuchi F, Shibata T, Watanabe Y. Altered sirtuin deacetylase gene expression in patients with a mood disorder. J Psychiatr Res. 2011; 45(8):1106–1112. [PubMed: 21349544]

Altamura AC, Boin F, Maes M. HPA axis and cytokines dysregulation in schizophrenia: potential implications for the antipsychotic treatment. Eur Neuropsychopharmacol. 1999; 10:1–4. [PubMed: 10647089]

Bahi A, Chandrasekar V, Dreyer JL. Selective lentiviral-mediated suppression of microRNA124a in the hippocampus evokes antidepressants-like effects in rats. Psychoneuroendocrinology. 2014; 46:78–87. [PubMed: 24882160]

Bak M, Silahtaroglu A, Moller M, Christensen M, Rath MF, Skryabin B, et al. MicroRNA expression in the adult mouse central nervous system. RNA. 2008; 14:432–444. [PubMed: 18230762]

Balakathiresan NS, Chandran R, Bhomia M, Jia M, Li H, Maheshwari RK. Serum and amygdala microRNA signatures of posttraumatic stress: fear correlation and biomarker potential. J Psychiatr Res. 2014; 57:65–73. [PubMed: 24998397]

Bavamian S, Mellios N, Lalonde J, Fass DM, Wang J, Sheridan SD, et al. Dysregulation of miR-34a links neuronal development to genetic risk factors for bipolar disorder. Mol Psychiatry. 2015

Benetatos L, Hatzimichael E, Londin E, Vartholomatos G, Loher P, Rigoutsos I, et al. The microRNAs within the DLK1-DIO3 genomic region: involvement in disease pathogenesis. Cellular and molecular life sciences : CMLS. 2013; 70:795–814. [PubMed: 22825660]

Berent D, Macander M, Szemraj J, Orzechowska A, Galecki P. Vascular endothelial growth factor A gene expression level is higher in patients with major depressive disorder and not affected by cigarette smoking, hyperlipidemia or treatment with statins. Acta Neurobiol Exp (Wars). 2014; 74(1):82–90. [PubMed: 24718046]

Berrettini W. Evidence for shared susceptibility in bipolar disorder and schizophrenia. Am J Med Genet C Semin Med Genet. 2003; 123C:59–64. [PubMed: 14601037]

Betel D, Koppal A, Agius P, Sander C, Leslie C. Comprehensive modeling of microRNA targets predicts functional non-conserved and non-canonical sites. Genome Biol. 2010; 11:R90. [PubMed: 20799968]

Beveridge NJ, Gardiner E, Carroll AP, Tooney PA, Cairns MJ. Schizophrenia is associated with an increase in cortical microRNA biogenesis. Mol Psychiatry. 2010; 15:1176–1189. [PubMed: 19721432]

Beveridge NJ, Tooney PA, Carroll AP, Gardiner E, Bowden N, Scott RJ, et al. Dysregulation of miRNA 181b in the temporal cortex in schizophrenia. Human molecular genetics. 2008; 17:1156–1168. [PubMed: 18184693]

Bierut LJ, Heath AC, Bucholz KK, Dinwiddie SH, Madden PA, Statham DJ, et al. Major depressive disorder in a community-based twin sample: are there different genetic and environmental contributions for men and women? Arch Gen Psychiatry. 1999; 56:557–563. [PubMed: 10359473]

Bradshaw NJ, Ogawa F, Antolin-Fontes B, Chubb JE, Carlyle BC, Christie S, et al. DISC1, PDE4B, and NDE1 at the centrosome and synapse. Biochem Biophys Res Commun. 2008; 377:1091–1096. [PubMed: 18983980]

Breslau N, Davis GC. Chronic stress and major depression. Arch Gen Psychiatry. 1986; 43:309–314. [PubMed: 2937384]

Broderick JA, Salomon WE, Ryder SP, Aronin N, Zamore PD. Argonaute protein identity and pairing geometry determine cooperativity in mammalian RNA silencing. RNA. 2011; 17:1858–1869. [PubMed: 21878547]

Bunney BG, Li JZ, Walsh DM, Stein R, Vawter MP, Cartagena P, et al. Circadian dysregulation of clock genes: clues to rapid treatments in major depressive disorder. Mol Psychiatry. 2015; 20:48–55. [PubMed: 25349171]

Burton PR, Clayton DG, Cardon LR, Craddock N, Deloukas P, Duncanson A, et al. Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. Nature. 2007; 447(7145):661–678. [PubMed: 17554300]

Cao X, Yeo G, Muotri AR, Kuwabara T, Gage FH. Noncoding RNAs in the mammalian central nervous system. Annu Rev Neurosci. 2006; 29:77–103. [PubMed: 16776580]

Cheng HY, Papp JW, Varlamova O, Dziema H, Russell B, Curfman JP, et al. microRNA modulation of circadian-clock period and entrainment. Neuron. 2007; 54:813–829. [PubMed: 17553428]

Cui Y, Xiao Z, Han J, Sun J, Ding W, Zhao Y, et al. MiR-125b orchestrates cell proliferation, differentiation and migration in neural stem/progenitor cells by targeting Nestin. BMC Neurosci. 2012; 13:116. [PubMed: 23016664]

Daban C, Vieta E, Mackin P, Young AH. Hypothalamic-pituitary-adrenal axis and bipolar disorder. Psychiatr Clin North Am. 2005; 28:469–480. [PubMed: 15826743]

Diler RS, Pan LA, Segreti A, Ladouceur CD, Forbes E, Cela SR, et al. Differential Anterior Cingulate Activity during Response Inhibition in Depressed Adolescents with Bipolar and Unipolar Major Depressive Disorder. J Can Acad Child Adolesc Psychiatry. 2014; 23:10–19. [PubMed: 24516473]

Drevets WC, Savitz J, Trimble M. The subgenual anterior cingulate cortex in mood disorders. CNS Spectr. 2008; 13:663–681. [PubMed: 18704022]

Ebert D, Ebmeier KP. The role of the cingulate gyrus in depression: from functional anatomy to neurochemistry. Biol Psychiatry. 1996; 39:1044–1050. [PubMed: 8780840]

Evans SJ, Choudary PV, Neal CR, Li JZ, Vawter MP, Tomita H, et al. Dysregulation of the fibroblast growth factor system in major depression. Proc Natl Acad Sci U S A. 2004; 101:15506–15511. [PubMed: 15483108]

Evans SJ, Choudary PV, Vawter MP, Li J, Meador-Woodruff JH, Lopez JF, et al. DNA microarray analysis of functionally discrete human brain regions reveals divergent transcriptional profiles. Neurobiol Dis. 2003; 14:240–250. [PubMed: 14572446]

Fan HM, Sun XY, Guo W, Zhong AF, Niu W, Zhao L, et al. Differential expression of microRNA in peripheral blood mononuclear cells as specific biomarker for major depressive disorder patients. J Psychiatr Res. 2014; 59:45–52. [PubMed: 25201637]

Fatemi SH, King DP, Reutiman TJ, Folsom TD, Laurence JA, Lee S, et al. PDE4B polymorphisms and decreased PDE4B expression are associated with schizophrenia. Schizophr Res. 2008; 101:36–49. [PubMed: 18394866]

Ferreira MA, O'Donovan MC, Meng YA, Jones IR, Ruderfer DM, Jones L, Fan J, Kirov G, Perlis RH, Green EK, Smoller JW, Grozeva D, Stone J, Nikolov I, Chambert K, Hamshere ML, Nimgaonkar VL, Moskvina V, Thase ME, Caesar S, Sachs GS, Franklin J, Gordon-Smith K, Ardlie KG, Gabriel SB, Fraser C, Blumenstiel B, Defelice M, Breen G, Gill M, Morris DW, Elkin A, Muir WJ, McGhee KA, Williamson R, MacIntyre DJ, MacLean AW, St CD, Robinson M, Van Beck M, Pereira AC, Kandaswamy R, McQuillin A, Collier DA, Bass NJ, Young AH, Lawrence J, Ferrier IN, Anjorin A, Farmer A, Curtis D, Scolnick EM, McGuffin P, Daly MJ, Corvin AP, Holmans PA, Blackwood DH, Gurling HM, Owen MJ, Purcell SM, Sklar P, Craddock N. Collaborative genome-wide association analysis supports a role for ANK3 and CACNA1C in bipolar disorder. Nat Genet. 2008; 40(9):1056–1058. [PubMed: 18711365]

Fleischhacker WW, Hinterhuber H, Bauer H, Pflug B, Berner P, Simhandl C, et al. A multicenter double-blind study of three different doses of the new cAMP-phosphodiesterase inhibitor rolipram in patients with major depressive disorder. Neuropsychobiology. 1992; 26:59–64. [PubMed: 1475038]

Fujino J, Yamasaki N, Miyata J, Sasaki H, Matsukawa N, Takemura A, et al. Anterior cingulate volume predicts response to cognitive behavioral therapy in major depressive disorder. J Affect Disord. 2015; 174:397–399. [PubMed: 25553399]

Gardiner E, Beveridge NJ, Wu JQ, Carr V, Scott RJ, Tooney PA, et al. Imprinted DLK1-DIO3 region of 14q32 defines a schizophrenia-associated miRNA signature in peripheral blood mononuclear cells. Mol Psychiatry. 2012; 17:827–840. [PubMed: 21727898]

Gibbons JL, Mc HP. Plasma cortisol in depressive illness. J Psychiatr Res. 1962; 1:162–171. [PubMed: 13947658]

Halsey LG, Curran-Everett D, Vowler SL, Drummond GB. The fickle P value generates irreproducible results. Nat Methods. 2015; 12(3):179–185. [PubMed: 25719825]

Haramati S, Navon I, Issler O, Ezra-Nevo G, Gil S, Zwang R, et al. MicroRNA as repressors of stress-induced anxiety: the case of amygdalar miR-34. J Neurosci. 2011; 31:14191–14203. [PubMed: 21976504]

Honda M, Kuwano Y, Katsuura-Kamano S, Kamezaki Y, Fujita K, Akaike Y, et al. Chronic academic stress increases a group of microRNAs in peripheral blood. PLoS One. 2013; 8:e75960. [PubMed: 24130753]

Huang, da W.; Sherman, BT.; Lempicki, RA. Bioinformatics enrichment tools: paths toward the comprehensive functional analysis of large gene lists. Nucleic Acids Res. 2009a; 37:1–13. [PubMed: 19033363]

Huang, da W.; Sherman, BT.; Lempicki, RA. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. Nat Protoc. 2009b; 4:44–57. [PubMed: 19131956]

Ilgen MA, Hutchison KE. A history of major depressive disorder and the response to stress. J Affect Disord. 2005; 86:143–150. [PubMed: 15935233]

Jacobsen A, Wen J, Marks DS, Krogh A. Signatures of RNA binding proteins globally coupled to effective microRNA target sites. Genome Res. 2010; 20:1010–1019. [PubMed: 20508147]

Jakovljevic M, Muck-Seler D, Pivac N, Crncevic Z. Platelet 5-HT and plasma cortisol concentrations after dexamethasone suppression test in patients with different time course of schizophrenia. Neuropsychobiology. 1998; 37:142–145. [PubMed: 9597670]

Katsuura S, Kuwano Y, Yamagishi N, Kurokawa K, Kajita K, Akaike Y, et al. MicroRNAs miR-144/144* and miR-16 in peripheral blood are potential biomarkers for naturalistic stress in healthy Japanese medical students. Neuroscience letters. 2012; 516:79–84. [PubMed: 22484483]

Kessler RC, Berglund P, Demler O, Jin R, Merikangas KR, Walters EE. Lifetime prevalence and age-of-onset distributions of DSM-IV disorders in the National Comorbidity Survey Replication. Arch Gen Psychiatry. 2005; 62:593–602. [PubMed: 15939837]

Kim AH, Reimers M, Maher B, Williamson V, McMichael O, McClay JL, et al. MicroRNA expression profiling in the prefrontal cortex of individuals affected with schizophrenia and bipolar disorders. Schizophr Res. 2010; 124:183–191. [PubMed: 20675101]

Kim D, Kim JW, Koo TH, Yun HR, Won SH. Shared and distinct neurocognitive endophenotypes of schizophrenia and psychotic bipolar disorder. Clin Psychopharmacol Neurosci. 2015; 13:94–102. [PubMed: 25912542]

Kishi T, Yoshimura R, Kitajima T, Okochi T, Okumura T, Tsunoka T, Yamanouchi Y, Kinoshita Y, Kawashima K, Fukuo Y, Naitoh H, Umene-Nakano W, Inada T, Nakamura J, Ozaki N, Iwata N. SIRT1 gene is associated with major depressive disorder in the Japanese population. J Affect Disord. 2010; 126(1–2):167–173. [PubMed: 20451257]

Kocerha J, Faghihi MA, Lopez-Toledano MA, Huang J, Ramsey AJ, Caron MG, et al. MicroRNA-219 modulates NMDA receptor-mediated neurobehavioral dysfunction. Proc Natl Acad Sci U S A. 2009; 106:3507–3512. [PubMed: 19196972]

Lachize S, Apostolakis EM, van der Laan S, Tijssen AM, Xu J, de Kloet ER, et al. Steroid receptor coactivator-1 is necessary for regulation of corticotropin-releasing hormone by chronic stress and glucocorticoids. Proc Natl Acad Sci U S A. 2009; 106:8038–8042. [PubMed: 19416907]

Lai CY, Yu SL, Hsieh MH, Chen CH, Chen HY, Wen CC, et al. MicroRNA expression aberration as potential peripheral blood biomarkers for schizophrenia. PLoS One. 2011; 6:e21635. [PubMed: 21738743]

Lewis BP, Burge CB, Bartel DP. Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets. Cell. 2005; 120:15–20. [PubMed: 15652477]

Li JZ, Vawter MP, Walsh DM, Tomita H, Evans SJ, Choudary PV, et al. Systematic changes in gene expression in postmortem human brains associated with tissue pH and terminal medical conditions. Human molecular genetics. 2004; 13:609–616. [PubMed: 14734628]

Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(−Delta Delta C(T)) Method. Methods. 2001; 25:402–408. [PubMed: 11846609]

Lohoff FW. Overview of the genetics of major depressive disorder. Curr Psychiatry Rep. 2010; 12:539–546. [PubMed: 20848240]

Makeyev EV, Zhang J, Carrasco MA, Maniatis T. The MicroRNA miR-124 promotes neuronal differentiation by triggering brain-specific alternative pre-mRNA splicing. Mol Cell. 2007; 27:435–448. [PubMed: 17679093]

Mayberg HS, Liotti M, Brannan SK, McGinnis S, Mahurin RK, Jerabek PA, et al. Reciprocal limbic-cortical function and negative mood: converging PET findings in depression and normal sadness. Am J Psychiatry. 1999; 156:675–682. [PubMed: 10327898]

McGirr A, Lipina TV, Mun HS, Georgiou J, Al-Amri AH, Ng E, et al. Specific Inhibition of Phosphodiesterase-4B Results in Anxiolysis and Facilitates Memory Acquisition. Neuropsychopharmacology. 2015

McGuffin P, Rijsdijk F, Andrew M, Sham P, Katz R, Cardno A. The heritability of bipolar affective disorder and the genetic relationship to unipolar depression. Arch Gen Psychiatry. 2003; 60:497–502. [PubMed: 12742871]

Mellios N, Huang HS, Grigorenko A, Rogaev E, Akbarian S. A set of differentially expressed miRNAs, including miR-30a-5p, act as post-transcriptional inhibitors of BDNF in prefrontal cortex. Human molecular genetics. 2008; 17:3030–3042. [PubMed: 18632683]

Millar JK, Pickard BS, Mackie S, James R, Christie S, Buchanan SR, et al. DISC1 and PDE4B are interacting genetic factors in schizophrenia that regulate cAMP signaling. Science. 2005; 310:1187–1191. [PubMed: 16293762]

Miller BH, Zeier Z, Xi L, Lanz TA, Deng S, Strathmann J, et al. MicroRNA-132 dysregulation in schizophrenia has implications for both neurodevelopment and adult brain function. Proc Natl Acad Sci U S A. 2012; 109:3125–3130. [PubMed: 22315408]

Moreau MP, Bruse SE, David-Rus R, Buyske S, Brzustowicz LM. Altered microRNA expression profiles in postmortem brain samples from individuals with schizophrenia and bipolar disorder. Biol Psychiatry. 2011; 69:188–193. [PubMed: 21183010]

Morgado AL, Xavier JM, Dionisio PA, Ribeiro MF, Dias RB, Sebastiao AM, et al. MicroRNA-34a Modulates Neural Stem Cell Differentiation by Regulating Expression of Synaptic and Autophagic Proteins. Mol Neurobiol. 2014

Muinos-Gimeno M, Espinosa-Parrilla Y, Guidi M, Kagerbauer B, Sipila T, Maron E, et al. Human microRNAs miR-22, miR-138-2, miR-148a, and miR-488 are associated with panic disorder and regulate several anxiety candidate genes and related pathways. Biol Psychiatry. 2011; 69:526–533. [PubMed: 21168126]

Mukherji S, Ebert MS, Zheng GX, Tsang JS, Sharp PA, van Oudenaarden A. MicroRNAs can generate thresholds in target gene expression. Nat Genet. 2011; 43:854–859. [PubMed: 21857679]

Mulert C, Juckel G, Brunnmeier M, Karch S, Leicht G, Mergl R, et al. Rostral anterior cingulate cortex activity in the theta band predicts response to antidepressive medication. Clin EEG Neurosci. 2007; 38:78–81. [PubMed: 17515172]

Nomura T, Kimura M, Horii T, Morita S, Soejima H, Kudo S, et al. MeCP2-dependent repression of an imprinted miR-184 released by depolarization. Human molecular genetics. 2008; 17:1192–1199. [PubMed: 18203756]

Numata S, Iga J, Nakataki M, Tayoshi S, Taniguchi K, Sumitani S, et al. Gene expression and association analyses of the phosphodiesterase 4B (PDE4B) gene in major depressive disorder in the Japanese population. Am J Med Genet B Neuropsychiatr Genet. 2009; 150B:527–534. [PubMed: 18785206]

Padmos RC, Hillegers MH, Knijff EM, Vonk R, Bouvy A, Staal FJ, et al. A discriminating messenger RNA signature for bipolar disorder formed by an aberrant expression of inflammatory genes in monocytes. Arch Gen Psychiatry. 2008; 65:395–407. [PubMed: 18391128]

Perkins DO, Jeffries CD, Jarskog LF, Thomson JM, Woods K, Newman MA, et al. microRNA expression in the prefrontal cortex of individuals with schizophrenia and schizoaffective disorder. Genome Biol. 2007; 8:R27. [PubMed: 17326821]

Pickard BS, Thomson PA, Christoforou A, Evans KL, Morris SW, Porteous DJ, et al. The PDE4B gene confers sex-specific protection against schizophrenia. Psychiatr Genet. 2007; 17:129–133. [PubMed: 17417055]

Pizzagalli D, Pascual-Marqui RD, Nitschke JB, Oakes TR, Larson CL, Abercrombie HC, et al. Anterior cingulate activity as a predictor of degree of treatment response in major depression: evidence from brain electrical tomography analysis. Am J Psychiatry. 2001; 158:405–415. [PubMed: 11229981]

Posner, M.; DiGirolamo, G. Executive attention: Conflict, target detection, and cognitive control. In: Parasuraman, R., editor. The Attentive Brain. MIT Press; 1998.

Purcell SM, Wray NR, Stone JL, Visscher PM, O'Donovan MC, Sullivan PF, et al. Common polygenic variation contributes to risk of schizophrenia and bipolar disorder. Nature. 2009; 460:748–752. [PubMed: 19571811]

Qi XR, Kamphuis W, Wang S, Wang Q, Lucassen PJ, Zhou JN, et al. Aberrant stress hormone receptor balance in the human prefrontal cortex and hypothalamic paraventricular nucleus of depressed patients. Psychoneuroendocrinology. 2013; 38:863–870. [PubMed: 23137715]

Rokavec M, Li H, Jiang L, Hermeking H. The p53/miR-34 axis in development and disease. J Mol Cell Biol. 2014; 6(3):214–230. [PubMed: 24815299]

Ruan CS, Zhou FH, He ZY, Wang SF, Yang CR, Shen YJ, et al. Mice deficient for wild-type p53-induced phosphatase 1 display elevated anxiety- and depression-like behaviors. Neuroscience. 2015; 293:12–22. [PubMed: 25732137]

Rueckert EH, Barker D, Ruderfer D, Bergen SE, O'Dushlaine C, Luce CJ, Sheridan SD, Theriault KM, Chambert K, Moran J, Purcell SM, Madison JM, Haggarty SJ, Sklar P. Cis-acting regulation

of brain-specific ANK3 gene expression by a genetic variant associated with bipolar disorder. Mol Psychiatry. 2013; 18(8):922–929. [PubMed: 22850628]

Salvadore G, Cornwell BR, Colon-Rosario V, Coppola R, Grillon C, Zarate CA Jr, et al. Increased anterior cingulate cortical activity in response to fearful faces: a neurophysiological biomarker that predicts rapid antidepressant response to ketamine. Biol Psychiatry. 2009; 65:289–295. [PubMed: 18822408]

Santarelli DM, Beveridge NJ, Tooney PA, Cairns MJ. Upregulation of dicer and microRNA expression in the dorsolateral prefrontal cortex Brodmann area 46 in schizophrenia. Biol Psychiatry. 2011; 69:180–187. [PubMed: 21111402]

Schratt GM, Tuebing F, Nigh EA, Kane CG, Sabatini ME, Kiebler M, et al. A brain-specific microRNA regulates dendritic spine development. Nature. 2006; 439:283–289. [PubMed: 16421561]

Sempere LF, Freemantle S, Pitha-Rowe I, Moss E, Dmitrovsky E, Ambros V. Expression profiling of mammalian microRNAs uncovers a subset of brain-expressed microRNAs with possible roles in murine and human neuronal differentiation. Genome Biol. 2004; 5:R13. [PubMed: 15003116]

Shepherd AM, Quide Y, Laurens KR, O'Reilly N, Rowland JE, Mitchell PB, et al. Shared intermediate phenotypes for schizophrenia and bipolar disorder: neuroanatomical features of subtypes distinguished by executive dysfunction. J Psychiatry Neurosci. 2015; 40:58–68. [PubMed: 25268788]

Shi W, Du J, Qi Y, Liang G, Wang T, Li S, et al. Aberrant expression of serum miRNAs in schizophrenia. J Psychiatr Res. 2012; 46:198–204. [PubMed: 22094284]

Sklar P, Ripke S, Scott LJ, Adreassen OA, Cichon S, Craddock N. et al. Large-scale genome-wide association analysis of bipolar disorder identifies a new susceptibility locus near ODZ4. Nat Genet. 2011; 43(10):977–983. [PubMed: 21926972]

Smalheiser NR, Lugli G, Zhang H, Rizavi H, Cook EH, Dwivedi Y. Expression of microRNAs and other small RNAs in prefrontal cortex in schizophrenia, bipolar disorder and depressed subjects. PLoS One. 2014; 9:e86469. [PubMed: 24475125]

Smoller JW, Finn CT. Family, twin, and adoption studies of bipolar disorder. Am J Med Genet C Semin Med Genet. 2003; 123C:48–58. [PubMed: 14601036]

Sun XY, Zhang J, Niu W, Guo W, Song HT, Li HY, et al. A preliminary analysis of microRNA as potential clinical biomarker for schizophrenia. Am J Med Genet B Neuropsychiatr Genet. 2015; 168:170–178. [PubMed: 25656957]

Tan LP, Seinen E, Duns G, de Jong D, Sibon OC, Poppema S, et al. A high throughput experimental approach to identify miRNA targets in human cells. Nucleic Acids Res. 2009; 37:e137. [PubMed: 19734348]

Tsai SJ, Hong CJ, Liou YJ, Chen TJ, Chen ML, Hou SJ, Yen FC, Yu YW. Haplotype analysis of single nucleotide polymorphisms in the vascular endothelial growth factor (VEGFA) gene and antidepressant treatment response in major depressive disorder. Psychiatry Res. 2009; 169(2):113–117. [PubMed: 19700204]

van der Laan S, Lachize SB, Vreugdenhil E, de Kloet ER, Meijer OC. Nuclear receptor coregulators differentially modulate induction and glucocorticoid receptor-mediated repression of the corticotropin-releasing hormone gene. Endocrinology. 2008; 149:725–732. [PubMed: 18006628]

Vreugdenhil E, Verissimo CS, Mariman R, Kamphorst JT, Barbosa JS, Zweers T, et al. MicroRNA 18 and 124a down-regulate the glucocorticoid receptor: implications for glucocorticoid responsiveness in the brain. Endocrinology. 2009; 150:2220–2228. [PubMed: 19131573]

Vythilingam M, Vermetten E, Anderson GM, Luckenbaugh D, Anderson ER, Snow J, et al. Hippocampal volume, memory, and cortisol status in major depressive disorder: effects of treatment. Biol Psychiatry. 2004; 56:101–112. [PubMed: 15231442]

Walker RM, Rybka J, Anderson SM, Torrance HS, Boxall R, Sussmann JE, et al. Preliminary investigation of miRNA expression in individuals at high familial risk of bipolar disorder. J Psychiatr Res. 2015; 62:48–55. [PubMed: 25708817]

Wan Y, Liu Y, Wang X, Wu J, Liu K, Zhou J, et al. Identification of differential microRNAs in cerebrospinal fluid and serum of patients with major depressive disorder. PLoS One. 2015; 10:e0121975. [PubMed: 25763923]

Wilczynska A, Bushell M. The complexity of miRNA-mediated repression. Cell Death Differ. 2015; 22:22–33. [PubMed: 25190144]

Winnay JN, Xu J, O'Malley BW, Hammer GD. Steroid receptor coactivator-1-deficient mice exhibit altered hypothalamic-pituitary-adrenal axis function. Endocrinology. 2006; 147:1322–1332. [PubMed: 16339206]

Xie X, Lu J, Kulbokas EJ, Golub TR, Mootha V, Lindblad-Toh K, et al. Systematic discovery of regulatory motifs in human promoters and 3' UTRs by comparison of several mammals. Nature. 2005; 434:338–345. [PubMed: 15735639]

Yamakuchi M, Ferlito M, Lowenstein CJ. miR-34a repression of SIRT1 regulates apoptosis. Proc Natl Acad Sci U S A. 2008; 105(36):13421–13426. [PubMed: 18755897]

Ye W, Lv Q, Wong CK, Hu S, Fu C, Hua Z, Cai G, Li G, Yang BB, Zhang Y. The effect of central loops in miRNA:MRE duplexes on the efficiency of miRNA-mediated gene regulation. PLoS One. 2008; 3(3):e1719. [PubMed: 18320040]

Yu JY, Chung KH, Deo M, Thompson RC, Turner DL. MicroRNA miR-124 regulates neurite outgrowth during neuronal differentiation. Exp Cell Res. 2008; 314:2618–2633. [PubMed: 18619591]

Yuan P, Tragon T, Xia M, Leclair CA, Skoumbourdis AP, Zheng W, et al. Phosphodiesterase 4 inhibitors enhance sexual pleasure-seeking activity in rodents. Pharmacol Biochem Behav. 2011; 98:349–355. [PubMed: 21296104]

Zeller E, Stief HJ, Pflug B, Sastre-y-Hernandez M. Results of a phase II study of the antidepressant effect of rolipram. Pharmacopsychiatry. 1984; 17:188–190. [PubMed: 6393150]

Zhou R, Yuan P, Wang Y, Hunsberger JG, Elkahloun A, Wei Y, Damschroder-Williams P, Du J, Chen G, Manji HK. Evidence for selective microRNAs and their effectors as common long-term targets for the actions of mood stabilizers. Neuropsychopharmacology. 2009; 34(6):1395–1405. [PubMed: 18704095]

Zhu Y, Kalbfleisch T, Brennan MD, Li Y. A MicroRNA gene is hosted in an intron of a schizophrenia-susceptibility gene. Schizophr Res. 2009; 109:86–89. [PubMed: 19264453]

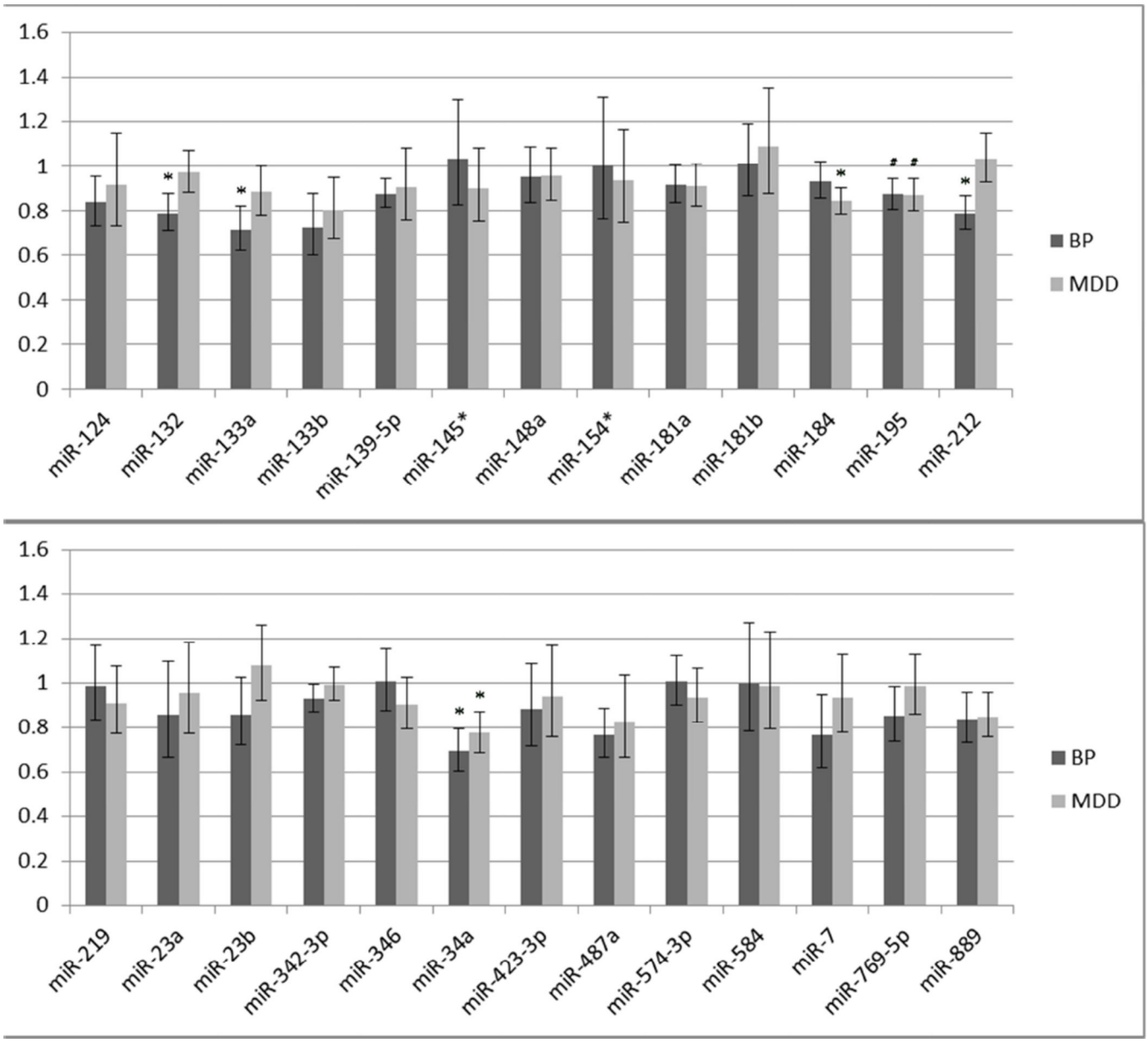

Figure 1. A subset of miRNAs validate as differentially expressed in AnCg of BP and/or MDD patients

Vertical axis represents linear fold change in BP or MDD cohorts versus control patients (BP: n=8; MDD: n=15; Control: n=14). Expression levels were measured using Taqman-chemistry-based qPCR using individual assays. Three miRNAs (*miR-132, 133a* and *212*) were dysregulated in BP, one (*miR-184*) was dysregulated in MDD and one miRNA—*miR-34a*, previously linked to schizophrenia—was dysregulated in both groups. All samples were run in technical triplicate with the snoRNA *RNU48* serving as a reference control. (*: $P < 0.05$, #: $P \leq 0.10$; error bars represent SEM.)

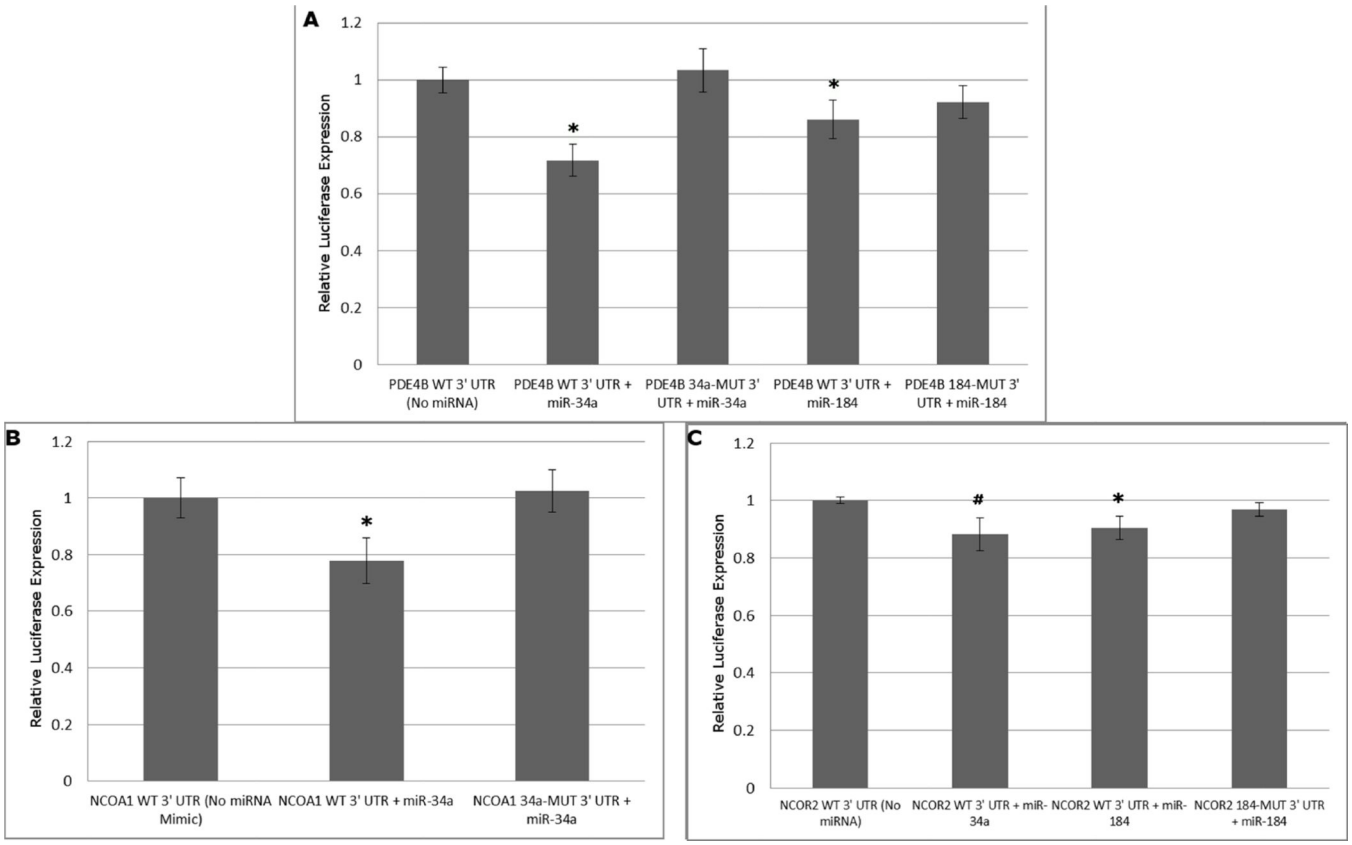

Figure 2. *PDE4B, NCOA1* and *NCOR2* are targets of miRNAs dysregulated in psychiatric illness

HEK293 cell cultures were plated and cotransfected with a miRNA mimic (either *miR-34a* or *miR-184*) and a firefly luciferase vector (Yu, Chung, 2008) containing either a wild-type 3' UTR (WT 3' UTR) or a 3' UTR mutagenized at a predicted miRNA binding site (34a-MUT or 184-MUT 3' UTR) (n=6/group). A vector encoding renilla luciferase was used to normalize luciferase activity. Significant reductions in luciferase activity were observed for *PDE4B* when cotransfected with either *miR-34a* or *miR-184* (**Fig. 2a**), *NCOA1* when cotransfected with *miR-34a* (**Fig. 2b**) and *NCOR2* when cotransfected with *miR-184* (**Fig. 2c**). Mutagenesis of the predicted binding sites was sufficient to relieve these 3' UTR constructs of miRNA-induced inhibition. Mutagenesis of sites in the *NCOR2* 3' UTR was not performed since this UTR showed no significant repression by *miR-34a*. (*: $P < 0.05$, #: $P \leq 0.10$; error bars represent SEM.)

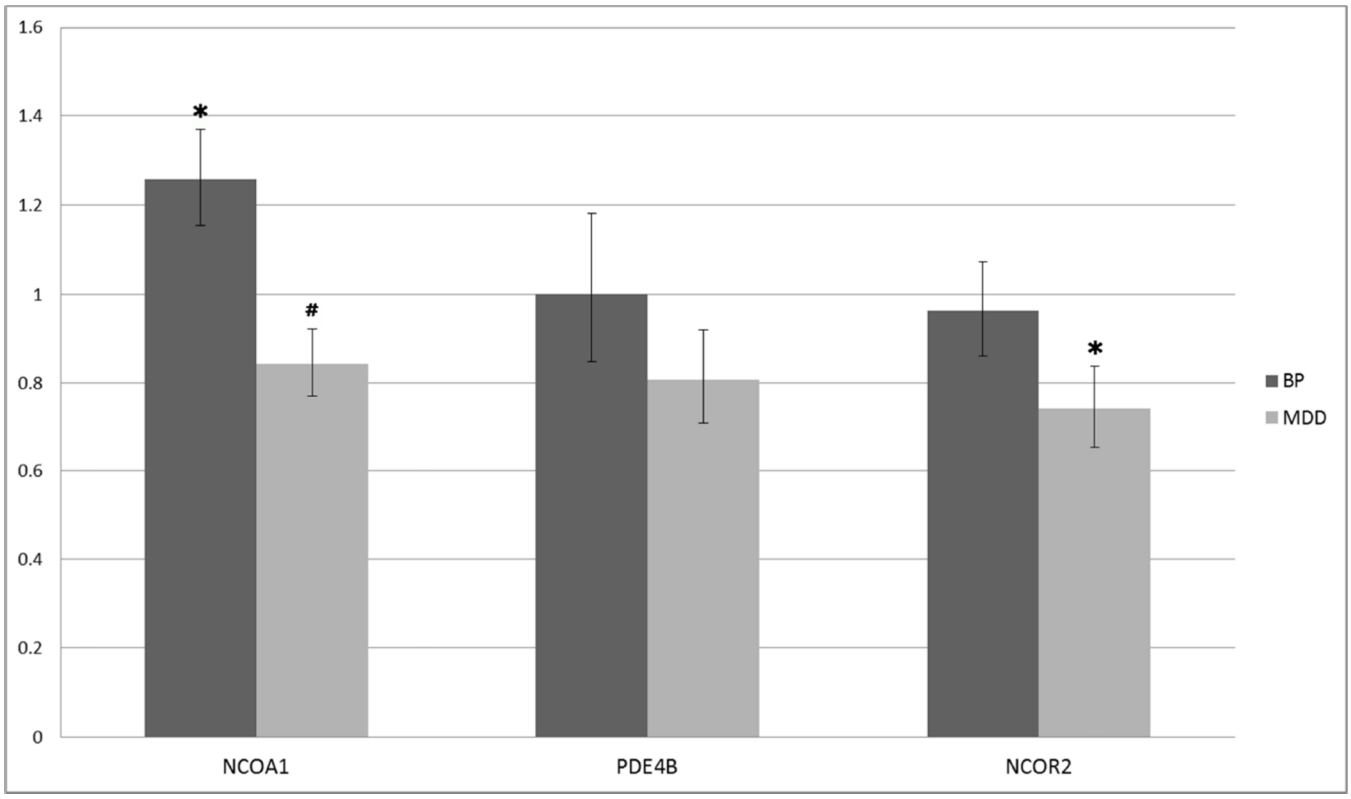

Figure 3. Steady-state mRNA levels of validated targets are altered in BP and MDD patients

The mRNA levels of *NCOA1* and *NCOR2* were dysregulated specifically in the BP and MDD cohorts, respectively. The same patient cohorts were used in both miRNA and mRNA expression analyses (BP: n=8; MDD: n=15 ; Control: n=14). Vertical axis represents linear fold change in BP or MDD patients versus controls. mRNA expression levels were analyzed using single-tube TaqMan qPCR assays specific to the genes of interest. Bars represent linear fold changes in BP and MDD cohorts compared to control patients with error bars representing SEM. (*: $P < 0.05$, #: $P \leq 0.10$)

Table 2

Validated mRNA targets of *miR-34a* with linkage to neuropsychiatric illness. Gene names in bold represent novel mRNA targets.

Gene	Gene Description		
GRM7	Metabotropic Glutamate Receptor 7	Synaptic transmission	GWAS (BP)(Fleischhacker et al., 1992, Zhou et al., 2009)
ANK3	Ankyrin G	Actin/spectrin adaptor protein	GWAS (BP)(Bavamian et al., 2015)
CACNB3	Voltage-Dependent L-Type Clacium Channel Subunit Beta-3	Synaptic transmission	GWAS (BP)(Bavamian et al., 2015)
VEGFA	Vascular Endothelial Growth Factor A	Growth factor signaling	Dysregulation of mRNA and protein levels, linkage analysis (MDD)(Berent et al., 2014, Tsai et al., 2009)
NCOA1	Nuclear Receptor Co-Activator 1	Modulation of gene transcription via glucocorticoid receptor	Dysregulation of mRNA levels (BP), modulation of stress responses and HPA axis activity. (Lachize et al., 2009, Winnay et al., 2006)
NCOR2	Nuclear Receptor Co-Repressor 2	Modulation of gene transcription via glucocorticoid receptor	Dysregulation of mRNA levels (MDD)
PDE4B	cAMP-specific 3',5'-cyclic Phosphodiesterase 4B	Regulates synaptic transmission via controlling cAMP levels	Dysregulation of protein levels (MDD), SNP (SZ and BP)(Fatemi et al., 2008, Pickard et al., 2007, Yuan et al., 2011)
SIRT1	Sirtuin 1	Histone deacetylase	Dysregulation of mRNA levels (BP, MDD)(Abe, 2011)