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MicroRNA expression profiling in the prefrontal cortex of individuals affected with schizophrenia and bipolar disorders

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Abstract

MicroRNAs (miRNAs) are a large family of small non-coding RNAs which negatively control gene expression at both the mRNA and protein levels. The number of miRNAs identified is growing rapidly and approximately one-third is expressed in the brain where they have been shown to affect neuronal differentiation, synaptosomal complex localization and synapse plasticity, all functions thought to be disrupted in schizophrenia. Here we investigated the expression of 667 miRNAs (miRBase v.13) in the prefrontal cortex of individuals with schizophrenia (SZ, N=35) and bipolar disorder (BP, N=35) using a real-time PCR-based Taqman Low Density Array (TLDA). After extensive QC steps, 441 miRNAs were included in the final analyses. At a FDR of 10%, 22 miRNAs were identified as being differentially expressed between cases and controls, 7 dysregulated in SZ and 15 in BP. Using in silico target gene prediction programs, the 22miRNAs were found to target brain specific genes contained within networks overrepresented for neurodevelopment, behavior, and SZ and BP disease development.

In an initial attempt to corroborate some of these predictions, we investigated the extent of correlation between the expressions of hsa-mir-34a, -132 and -212 and their predicted gene

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Contributors

A.H.K. and O. M. performed the experimental work. M.R., B.M., J.M. and E. G.C.J. undertook all statistical analyses and helped with their interpretation. V.W. performed the in silico analyses. VIV designed the study and wrote the first draft of the manuscript. B.P.R. and K.S.K. contributed to the final writing of the manuscript. All authors contributed to and have approved the final manuscript.

Conflict of interest

The authors declare no financial interests or potential conflicts of interest.

targets. mRNA expression of tyrosine hydroxylase (TH), phosphogluconate dehydrogenase (PGD) and metabotropic glutamate receptor 3 (GRM3) was measured in the SMRI sample. Hsa-miR-132 and -212 were negatively correlated with TH ($p=0.0001$ and 0.0017) and with PGD ($p=0.0054$ and 0.017 , respectively).

Keywords

MiRNA; Schizophrenia; Bipolar; Gene; Expression; TLDA

1. Introduction

Both schizophrenia (SZ, MIM 181500) and bipolar disorder (BP, MIM 125480) are common and debilitating psychiatric illnesses with a lifetime prevalence of 0.5–1% and 0.8–2.6% respectively. The etiology of SZ and BP is currently unknown, however in conjunction with environmental and developmental factors (Jablensky et al., 1992; Kendler et al., 1994; Kendler and Diehl, 1993), consistent evidence for a substantial genetic component (Kato et al., 2005) has been shown, with some shared between both diseases (Berrettini, 2003; Purcell et al., 2009a).

Protein-coding genes have long been the focus of research in disease genetics, but efforts to identify replicable protein-coding risk loci in SZ (Shi et al., 2009a; Stefansson et al., 2009b) and BP (Sklar et al., 2008) remain elusive. Current GWAS studies have also been unable to establish a uniform set of protein-coding genes exhibiting a clear etiology in disease (Purcell et al., 2009b; Shi et al., 2009b; Stefansson et al., 2009a). Additionally, genetic association does not appear to reflect DNA variants with any obvious effect on protein structure (Bray, 2008) for several of the best supported SZ susceptibility genes identified to date (e.g. DTNBP1 and NRG1).

MiRNAs are a large family of small non-coding RNAs which negatively control gene expression at both the mRNA and protein levels (Lai, 2002). Most miRNAs are processed from a Pol II transcript called a primary miRNA (pri-miRNA), which is further processed to a smaller hairpin-like structure called a precursor miRNA (pre-miRNA). The terminal loop of the pre-miRNA is then cleaved, generating a miRNA/miRNA* duplex and one strand is preferentially loaded into the RNA induced silencing complex (RISC) (Filipowicz et al., 2008) leading to mRNA degradation and/or translational repression. MiRNAs control mRNA stability and translation by binding to sequence motifs in the 3'-UTR of target mRNAs (Valencia-Sanchez et al., 2006). This binding does not require complete complementarity except for nucleotides 2–8 (the “seed region”) at the miRNA's 5' end. Although the majority of miRNA/ mRNA interactions require perfect complementarity at the seed region, there are some exceptions to this rule, which complicates miRNA target predictions (Lai, 2004).

Microarray studies have revealed that individual miRNAs can affect the expression of multiple genes (Krichevsky et al., 2006), suggesting that miRNAs can have pleiotropic effects on cellular processes. A growing number of miRNAs are discovered every year (Griffiths-Jones et al., 2006) and approximately one-third is expressed in the brain (Sempere

et al., 2004) where they have been shown to be involved in maintaining brain function (Fiore et al., 2008). In vitro studies demonstrate that miRNAs are localized to the synaptosomal complex (Lugli et al., 2005) where they affect neuronal differentiation (Vo et al., 2005) and modulate synapse plasticity (Schratt et al., 2006), both of which have been implicated in SZ and BP (Eastwood and Harrison, 2001; Vawter et al., 2002). Thus, miRNAs are likely candidates in neurodegenerative disorders (Kuss and Chen, 2008) and recent studies have attempted to evaluate the impact of miRNAs in SZ etiology at both genetic (Rogaev, 2005; Hansen et al., 2007; Burmistrova et al., 2007) and expression (Burmistrova et al., 2007; Beveridge et al., 2008; Perkins et al., 2007) levels.

Postmortem brain tissue has been successfully used to identify transcriptional dysregulation of candidate genes in SZ and BP (Harrison and Weinberger, 2005). Here, we evaluated the expression of 667 miRNAs in the postmortem prefrontal cortex of patients with SZ and BP disorder.

2. Materials and methods

2.1. Postmortem brain tissue

The Stanley Medical Research Institute (SMRI) provided 200 mg of postmortem brain tissue from the dorsolateral prefrontal cortex (DLPFC, Brodmann's area 46) (Torrey et al., 2000). The sample demographics are described in Suppl. Table 1. Exclusion criteria included: (1) brain pathology, (2) history of pre-existing CNS disease, (3) poor RNA quality, (4) IQ<70, (5) age<30 and (6) substance abuse within one year of death.

2.2. RNA isolation and quantification

Total RNA containing the small RNA fraction was isolated from 100 mg of tissue using the miRvana-Paris Kit (Ambion, Austin, Texas) following manufacturer's recommendations. RNA quality and concentration were measured on the 21000 Bioanalyzer (Agilent, Santa Clara, CA). Average RNA integrity number (RIN) was 7.2. The tissue for one BP sample was not received and 1 CON sample gave poor RNA quality, both of which were excluded from further analyses.

2.3. MiRNA reverse transcription

cDNA synthesis for TLDA was performed according to manufacturer's recommendation (ABI). Briefly, RNA was reverse transcribed using the MicroRNA reverse transcription kit (ABI) in combination with the stem-loop Megaplex primer pool (ABI). 3 µl of total RNA (33.3 ng/µl) was combined with 0.8 µl RT primer mix (10×), 0.8 µl RT buffer (10×), 1.5 µl MultiScribe Reverse Transcriptase (10 U/µl), 0.2 µl dNTPs with dTTP (0.5 mM each), 0.9 µl MgCl₂ (3 mM) and 0.1 µl RNase inhibitor (0.25 U/µl) in a total reaction volume of 7.5 µl. Reactions were run on an Eppendorf Mastercycler (Eppendorf, Westbury, NY) in a 384-well plate for 40 cycles at 16 °C for 2 min, 42 °C for 1 min, 50 °C for 1 s, and 85 °C for 5 min.

cDNA synthesis for the single tube miRNA reactions was performed using the TaqMan MicroRNA reverse transcription kit (ABI). 10 ng of RNA (2 ng/µl) was combined with 3.0 µl 5× RT primer, 0.15 µl 100 mM dNTPs, 1.0 µl MultiScribe Reverse Transcriptase (50 U/

μl), 1.5 μl 10× RT buffer and 0.19 μl RNase inhibitor (20 U/μl) in a total reaction volume of 15 μl. Reactions were run on an Eppendorf Mastercycler (Eppendorf) in a 96-well plate for 40 cycles at 16 °C for 30 min, 42 °C for 30 min and 85 °C for 5 min.

2.4. MiRNA expression detection

Prior to detecting miRNA expression, the cDNA for the TLDA array was pre-amplified following ABI's recommendations, allowing for increased detection sensitivity while preserving the miRNA (Cogswell et al., 2008b; Mestdagh et al., 2008; Tang et al., 2006) expression profile (Suppl. Fig. 1). The reactions were diluted with 75 μl of 0.1× TBE and stored at −20 °C. 9 μl of product and 450 μl of TaqMan PCR Master Mix, was combined with 441 μl nuclease free water, mixed and centrifuged for 30 s. 100 μl was loaded into each port of the appropriate 384 well TLDA array, centrifuged twice at 1200 rpm in a Sorvall Legend centrifuge (Thermo Scientific, USA), and sealed with a micro-fluidic card staker (ABI). The arrays were run on the 7900HT Real-Time PCR System according to manufacturer's protocol. Raw Cq values (RDML guidelines, <http://www.rdm.org> (Lefever et al., 2009)) were calculated using the SDS software v.2.3 with automatic baseline settings at a threshold of 0.2.

The single tube miRNA expressions were detected using TaqMan chemistry according to the manufacturer's protocol (ABI). 1.33 μl of cDNA (diluted 1:15) was combined with 1.0 μl 20× TaqMan MicroRNA assay and 10.0 μl 2× TaqMan Universal PCR MasterMix, No AmpErase UNG in a final volume of 20 μl. The reactions were run in triplicate in a 384-well plate on the 7900HT Real-Time PCR System according to manufacturer's recommendations (ABI).

2.5. MiRNA PCR expression normalization

Under our experimental conditions, the three reference genes contained on the A and B TLDA arrays did not maintain a consistent ratio across samples (Suppl. Fig. 2). Therefore, we normalized our data using the median polish procedure (Bolstad et al., 2003), a method similar to that employed by others to evaluate global miRNA expression (Abu-Elneel et al., 2008). A more detailed description of the method is given in the Supplementary (S2a) material.

2.6. Quality control (QC)

Potential technical artifacts, such as plate effects (Sundberg et al., 2006), were evaluated by plotting the sample profile correlations which were invariant under this normalization. Two bands of low correlations were observed, which we interpreted as plate effects independent of diagnosis (Suppl. Fig. 3). Samples falling within these bands were excluded from further analyses as they also clustered separately from the main group (Suppl. Fig. 4). Thus, the final sample was reduced to 29 SZ, 27 BP and 31 CONT.

2.7. Gene expression

mRNA samples (300 ng) were reverse transcribed into cDNA using the high capacity cDNA Kit from ABI following the manufacturer's instructions. TH, PGD and GRM3 gene expressions were measured by Taqman chemistry on the 7900HT instrument according to

the manufacturer's recommendation and normalized against the IPO8 reference control using the 2^{-C_t} algorithm.

2.8. Statistical methods

The primary analyses were conducted using the `rlm` function in the R open-source statistical language (www.r-project.org) and the exploratory analyses were conducted using Robust Multiple Regression with Huber's method as implemented in the NCSS (<http://www.ncss.com>) software package. MiRNA variance was estimated using an F-test with corrected degrees of freedom and the Spearman (ρ) coefficient was used to estimate correlations of miRNA expressions. Correction for multiple testing was accomplished using the Benjamini–Hochberg (Benjamini and Hochberg, 1995) False Discovery Rate (FDR) at 10%. A complete list of p-values and respective q-values for the entire miRNA data set is provided as well (Suppl. Table. 2). The significantly expressed SZ and BP miRNAs were correlated using Spearman ρ coefficient.

The single tube miRNA expression data were log transformed or raised to the power of 1/3 (Suppl. Table 3) to approximate a normal distribution and analyzed within a multiple regression framework with disease as the main factor and pH and sex included as covariates. Gene targets for the differentially expressed miRNAs were predicted in house by the miRanda algorithm (Enright et al., 2004) (V.3) using default parameters. For the correlation analysis, the expression of miRNAs and their respective gene targets were normalized and log transformed or raised to the power of 1/3 (Suppl. Table 4). The expression values were fitted into an analysis of co-variance (ANCOVA) model with pH, age, RIN, sex and disease status as covariates. The effect of these potential covariates was regressed out and the residuals were then correlated using the Pearson product moment.

3. Results

3.1. Overall performance

Each individual in the SMRI sample was evaluated for the expression of 667 miRNAs. Of these, 226 miRNAs were excluded from further analyses due to lack of amplification, very low expression ($C_q > 35$), or poor amplification efficiency across samples (missing expression values $> 80\%$). 441 miRNAs were included in all subsequent analyses (Suppl. Table 5) and based on their average expression were arbitrarily classified as highly (C_q 14–20), moderately (C_q 21–26), or low (C_q 27–34) expressed (Suppl. Table 6). Being a global miRNA evaluation, it is reasonable to expect that not all miRNAs are brain expressed.

3.2. SMRI demographics

Together with disease status, the SMRI provided additional information regarding potential confounders described in Supplementary S2b. Upon inspection, only PMI, RI and antipsychotics showed deviation from a normal distribution, which were log transformed to approximate normality. In our analyses, none of the covariates showed consistent effects on miRNA expression. When fitting a regression model to a small sample size, the number of estimated parameters cannot be large, therefore in all analyses, only sex and pH were included as technical covariates.

3.3. Detection of differentially expressed miRNAs in SZ and BP cases

Our main objective was to evaluate expression differences between SZ and BP cases and controls for all known human miRNAs using the TLDA high throughput real-time qPCR approach. The T-score distribution from the linear model showed deviation from the expected normal distribution (Fig. 1), and after controlling the FDR at 10%, 22 miRNAs were identified as significantly dysregulated in SZ and BP (Table 1). Notably, hsa-miR-7, -212 and -132, which were previously reported to be dysregulated in the prefrontal cortex of individuals with SZ and schizoaffective disorder (Perkins et al., 2007), were also dysregulated in our sample.

3.4. SZ cases

Seven miRNAs were differentially expressed in the SZ group. Of these, hsa-miR-34a, located on chromosome 1 (1p36.22), was the most significantly differentially expressed ($p=0.00004$) and in contrast to a previous study showing high expression (Lim et al., 2005), this miRNA was moderately expressed (average Cq=21.8). Hsa-miR-132 and -132* are processed from the same precursor and together with hsa-miR-212, form a cluster located on the reverse strand of chromosome 17 (17p13.3). Hsa-miR-132 and -212 were previously reported to be expressed in brain and our data confirm this observation (Lu et al., 2005). Hsa-miR-132 was highly expressed (average Cq=17.5) and hsa-miR-212 was moderately expressed at an (average Cq of 23). Both miRNAs were highly correlated (Spearman's $\rho=0.87$), supporting previous observations for coordinated expression of clustered miRNAs (Lee and Kim, 2004; Lee et al., 2004; Sempere et al., 2004). Hsa-miR-132* however, was expressed at a lower level (average Cq=27.18) and was less correlated with hsa-miR-132 (Spearman's $\rho=0.65$) and hsa-miR-212 ($\rho=0.47$). Hsa-miR-7 consists of three members located on three chromosomes; hsa-miR-7-1 (9q21.32), hsa-miR-7-2 (15q21.6) and hsa-miR-7-3 (19p13.3). The hsa-miR-7 family showed moderate expression (average Cq=23.1), but since the mature sequence of these three miRNAs is identical, individual expression of each member could not be separated. Finally, hsa-miR-544 was low (Cq=31.35) and hsa-miR-154* was moderately (Cq=26.31) expressed. Hsa-miR-154* was the only miRNA showing expression differences in both SZ and BP.

3.5. BP cases

In the BP group, 15 miRNAs were observed to be differentially expressed. Seven were over-expressed and 8 were under-expressed (Table 1). The over-expressed miRNAs were all moderately to highly expressed and showed significant correlation with each other (Table 2A). In contrast, the under-expressed miRNAs were moderately to low expressed and did not show any significant inter-correlations (Table 2B). Of all correlated miRNAs, only two are known to cluster together, hsa-miR-154* and -889 on chr.14 (14q32.2). However in contrast to the clustered miRNAs observed in the SZ group, hsa-miR-154* and -889 were only modestly correlated with each other (Spearman's $\rho=0.35$).

3.6. TLDA validation

All 22 miRNAs differentially expressed from the TLDA approach were validated in single tube real-time PCR reactions. One miRNA, hsa-miR-573, was not amplified and therefore

excluded from the validation analyses. The remaining 21 miRNA expressions were normalized by the 2^{-C_t} approach using RNU48 as an endogenous control. Nine miRNAs were successfully validated, 5 in SZ and 4 in BP (Suppl. Table 7). Interestingly, 3 of the SZ specific miRNAs were also significantly expressed in the BP group and 2 of the BP specific miRNAs were also significantly expressed in the SZ group. This observation is not surprising considering the proposed common etiology between SZ and BP disorders (Berrettini, 2003; Purcell et al., 2009a). The discrepancy between the multiplex and singly validated miRNA data could be explained by biological (heterogeneity) or technical (PCR conditions or pre-amplification) differences. Additional expression studies will be needed to address such issues. However, many of the validated miRNAs reported here have also been previously reported in other studies using different platforms and/ or samples.

3.7. Analysis of miRNA variances

Although miRNAs tend to be dysregulated among individuals with SZ or BP, a limited number of miRNAs (22/441 or 5%) demonstrated a consistent direction of altered expression, e.g. the same miRNA following a similar direction of expression. This is not surprising considering the multigenic etiology of SZ and BP. Moreover, when miRNA variances between groups were compared, many of the miRNAs showing significant variance differences (Suppl. Table 8) were present in both disease groups (Fig. 2), thereby supporting the shared genetic risk in SZ and BP that others have observed (Berrettini, 2003; Purcell et al., 2009a).

3.8. Exploratory analyses

Exploratory analyses were performed to test changes in miRNA expression for disease duration (Dean et al., 2007), smoking (Zammit et al., 2003), suicide (Pompili et al., 2007), and antipsychotic treatment as these traits were previously shown to affect expression of SZ candidate genes. The only significant effect was observed for hsa-miR-193b* ($p=0.00002$) when suicide was analyzed in interaction with disease. Antipsychotic treatment did not show any systematic effect on miRNA expression with hsa-miR-218-2* ($p=0.000012$) expression affected in the BP group only. A probable explanation is that in the SMRI sample, antipsychotic treatment is reported as a generalized measure (fluphenazine equivalents). Since different drugs have different (if not opposing) affects on miRNA expression, the individual drug effect is likely to be confounded.

3.9. In silico analyses

Using miRanda (John et al., 2004) we identified 3171 predicted gene targets for the 5 validated miRNAs differentially expressed in the SZ group and 2666 predicted gene targets for the 4 validated miRNAs differentially expressed in the BP group (Suppl. Table 9A and 9B). The nature of these gene targets was then evaluated using the IPA web tool. In IPA, our gene dataset was used as the reference set to minimize experimental or literature bias. The most enriched functional group of genes amongst the differentially expressed miRNA targets (Suppl. Fig. 5A and 5B) were genes forming networks related to nervous system function and disease, including SZ and BP.

In an initial attempt to corroborate some of these predictions, we investigated the extent of correlation between the expressions of hsa-miR-34a, -132 and -212 and their predicted gene targets. We focused on these miRNAs because they were highly significant in our study and previous reports implicated the min SZ and autism. Based on miRanda and IPA, three disease relevant gene targets for hsa-miR-34a, -132 and -212 were tyrosine hydroxylase (TH), metabotropic glutamate receptor 3 (GRM3) and phosphogluconate dehydrogenase (PGD). We measured the mRNA expression of TH, GRM3 and PGD in the SMRI postmortem sample and correlated these with their respective miRNAs: hsa-miR-34a with TH and PGD; hsa-miR-132 with TH and GRM3; and hsa-miR-212 with TH and GRM3. Since miRNAs primarily act to negatively regulate gene expression, miRNA over-expression should lead to downregulation of their gene targets (Wang and Wang, 2006) resulting in a negative correlation. Hsa-miR-34a did not show significant correlations with its predicted target genes. However, hsa-miR-132 was negatively correlated with TH, $r = -0.41$ ($p = 0.0001$) and PGD, $r = -0.29$ ($p = 0.0054$) and hsa-miR-212 with TH $r = -0.35$, ($p = 0.0017$) and PGD $r = -0.22$, ($p = 0.017$), respectively (Suppl. Fig. 6). Although PGD was not computationally predicted to be targeted by these two miRNAs, this observation reflects previous experimental studies demonstrating physical interaction between miRNA and genes that are not bioinformatically predicted as miRNA targets (Beitzinger et al., 2007).

4. Discussion

MiRNAs have key roles in regulating gene expression and brain development, and thus are likely genetic factors contributing to the etiology of psychiatric disorders. In this study, we conducted an evaluation of miRNA expression in postmortem tissues from SZ and BP cases. Of the 441 miRNAs analyzed, we identified 7 to be differentially expressed in SZ and 15 to be differentially expressed in BP, which constitutes ~5% of miRNAs studied. Similar observations were made in other studies of SZ (Perkins et al., 2007) and autistic (Abu-Elneel et al., 2008) cases. Although few miRNAs showed consistent unidirectional expression differences between cases and controls, we observed a large number of miRNAs with significant variance differences in SZ and BP, many of which were shared by both disease groups. Thus we, as others (Abu-Elneel et al., 2008; Perkins et al., 2005), argue that the development of SZ and BP is likely to reflect two classes of miRNAs: the first class consisting of miRNAs showing consistent unilateral expression across all cases in the disease groups, and the second class showing much greater individual variability of expression.

Several studies have attempted to address differential expression of miRNAs in the etiology of SZ. Perkins et al. (2007) were the first to evaluate miRNA expression in a postmortem brain sample consisting of 13 individuals with SZ and 2 individuals with schizoaffective disorder. They identified 16 miRNAs dysregulated in cases, two of which, hsa-miR-7 and -212, were also differentially expressed in our study. In two separate studies, Beveridge et al. (2008; 2009) observed 26 differentially expressed miRNAs in the DLPFC of SZ cases, including hsa-miR-7. In a fourth study of autistic cases (Abu-Elneel et al., 2008), 28 miRNAs were shown to be dysregulated across 9 autistic samples, including hsa-miR-7 and -132.

In addition to being dysregulated in SZ and autistic cases, hsa-miR-7 has been shown to be involved in the development of Parkinson's disorder by repressing the expression of alpha synuclein (α -Syn), a protein that accumulates in the nigral dopaminergic neurons (Junn et al., 2009). Hsa-miR-7 was also shown to be involved in the development of glioblastoma by repressing the epidermal growth factor receptor (*EGFR*) gene and the *AKT1* signaling pathway (Kefas et al., 2008). The repressing effect of hsa-miR-7 on *AKT1* is noteworthy since AKT1 has been shown to be associated with BP (Toyota et al., 2003) and with SZ across multiple populations (Bajestan et al., 2006; Emamian et al., 2004; Ikeda et al., 2004; Schwab et al., 2005; Thiselton et al., 2008).

Hsa-miR-132 and -212 expressions have been reported to be dysregulated in Alzheimer's disease (Cogswell et al., 2008a), suggesting a broader impact of these miRNAs on diseases of brain development. The predicted hsa-miR-132 and miR-132* gene targets interact with genes known to be involved in SZ (*GRIN1*, *GRIN2A*, *GRIN2B*, brain-derived neurotrophic factor (*BDNF*) and Dopamine receptor 1 (*DRD1*)). *GRIN1*, *GRIN2A*, and *GRIN2B* encode different subunits of the NMDA receptor, the dysfunction of which is a major etiological hypothesis of SZ pathophysiology (Javitt, 2007). BDNF mRNA levels have been shown to be significantly reduced in the DLPFC of schizophrenics (Weickert et al., 2003). *DRD1* is thought to have a role in the regulation of cognitive functions in the DLPFC (Missale et al., 2006) and is also thought to be involved in the action of the atypical antipsychotic clozapine used in the treatment of schizophrenia (Stone et al., 2007).

Finally, several reports have linked CNVs to the genetic risk of SZ (Kirov et al., 2008; Stefansson et al., 2008; Stone et al., 2008; Xu et al., 2008). Using a database of genomic variants (<http://projects.tcag.ca/variation/>), we identified 7 miRNAs from our list of dysregulated miRNAs mapping to CNV regions (Suppl. Table 10). Although none of these CNVs on chromosomes 1, 9, and 17 have been reported to be associated with SZ, a robust relationship between disease (cancer) phenotype and miRNAs located in CNVs has been established (Zhang et al., 2006), suggesting that a possible association between CNVs and miRNAs differentially expressed in SZ and BP may exist as well.

5. Conclusions

In this study we evaluated 667 miRNAs, 441 of which showed reliable expression in the SMRI postmortem brain sample. This is the largest set of miRNAs evaluated to date, hence it is not surprising that we observed a greater number of miRNAs (two-thirds) to be expressed in brain than previously reported (Sempere et al., 2004). Twenty two miRNAs were found to be differentially expressed between cases and controls, three of which, hsa-miR-7, -132, and -212 were previously reported to be dysregulated in SZ. Furthermore, in silico predicted gene targets for these miRNAs were found to be overrepresented in networks relevant to SZ and BP disorders. Going further, we observed a negative correlation between the expression of miRNAs and their predicted gene targets, providing preliminary support for our computational predictions. Thus, miRNAs are likely to contribute to disease development by modulating expression of previously identified gene candidates for SZ and BP and our study provides additional evidence for miRNA involvement in the etiology of

disease. To our knowledge, we are also the first to report differentially expressed miRNAs in BP disorder.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at [doi:10.1016/j.schres.2010.07.002](https://doi.org/10.1016/j.schres.2010.07.002).

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Fig. 1. Q-Q plots of the T-scores distribution within the SZ group. 1B. Q-Q plots of the T-scores distribution within the BP group. T-scores are obtained from the linear model.

Fig. 2.
A Venn diagram showing the number of disease specific and shared miRNAs with significant variances for the two diagnostic groups.

Significantly expressed miRNAs in the SZ and BP groups compared to controls at FDR of 10%. The miRNAs highlighted in the SZ group were reported in previous studies. See text for more details. The negative values of the T-scores reflect up- or down-regulation of specific miRNA expression.

Table 1

Status	MIRNA	Aver. Cq	$\beta(0)$	SE	T-value	P-value	Q-value
SZ	hsa-miR-34a	21.82	0.385	0.093	4.12	0.00004	0.018
SZ	hsa-miR-132*	29.14	0.364	0.092	3.98	0.00016	0.024
SZ	hsa-miR-132	17.47	0.212	0.053	3.97	0.00016	0.024
SZ	hsa-miR-212	23.04	0.232	0.064	3.65	0.00047	0.048
SZ	hsa-miR-544	31.35	0.839	0.243	3.46	0.00054	0.048
SZ	hsa-miR-7	23.1	0.241	0.069	3.50	0.00078	0.054
SZ	hsa-miR-154*	26.31	0.511	0.153	3.33	0.00086	0.054
BP	hsa-miR-504	24	0.710	0.169	4.19	0.00003	0.007
BP	hsa-miR-454*	30.53	-0.925	0.226	-4.10	0.00004	0.007
BP	hsa-miR-29a	17.64	-0.297	0.073	-4.07	0.00005	0.007
BP	hsa-miR-520c-3p	30.11	-0.617	0.165	-3.75	0.00018	0.020
BP	hsa-miR-140-3p	25.14	-0.473	0.137	-3.47	0.00053	0.047
BP	hsa-miR-145*	26.6	0.572	0.171	3.35	0.00080	0.052
BP	hsa-miR-767-5p	26.87	-0.329	0.100	-3.29	0.00102	0.052
BP	hsa-miR-22*	23.19	0.465	0.142	3.27	0.00106	0.052
BP	hsa-miR-145	17.83	0.571	0.183	3.13	0.00177	0.066
BP	hsa-miR-874	21.9	-0.391	0.125	-3.12	0.00181	0.066
BP	hsa-miR-133b	24.98	0.359	0.116	3.11	0.00190	0.066
BP	hsa-miR-154*	26.31	0.501	0.162	3.10	0.00195	0.066
BP	hsa-miR-32	29.18	-0.724	0.235	-3.08	0.00209	0.066
BP	hsa-miR-573	29.03	-0.939	0.308	-3.05	0.00227	0.067
BP	hsa-miR-889	24.83	0.433	0.147	2.95	0.00321	0.089

Table 2

A

Correlation matrix of miRNAs over-expressed in the BP group. Spearman's p coefficients in bold are statistically significant at p<0.05.

Variables	hsa-miR-504	hsa-miR-145	hsa-miR-145*	hsa-miR-22*	hsa-miR-133b	hsa-miR-154*	hsa-miR-889
hsa-miR-504	1	0.420	0.503	0.608	0.512	0.611	0.633
hsa-miR-145	0.420	1	0.840	0.427	0.550	0.230	0.276
hsa-miR-145*	0.503	0.840	1	0.493	0.451	0.397	0.364
hsa-miR-22*	0.608	0.427	0.493	1	0.344	0.554	0.550
hsa-miR-133b	0.512	0.550	0.451	0.344	1	0.141	0.485
hsa-miR-154*	0.611	0.230	0.397	0.554	0.141	1	0.355
hsa-miR-889	0.633	0.276	0.364	0.550	0.485	0.355	1

B

Correlation matrix of miRNAs under-expressed in BP group. Spearman's p coefficients in bold are statistically significant at p<0.05.

Variables	hsa-miR-140-3p	hsa-miR-29a	hsa-miR-32	hsa-miR-874	hsa-miR-454*	hsa-miR-520c-3p	hsa-miR-573	hsa-miR-767-5p
hsa-miR-140-3p	1	0.127	0.288	0.062	0.392	0.051	-0.067	-0.057
hsa-miR-29a	0.127	1	0.163	0.017	0.244	0.165	-0.067	0.245
hsa-miR-32	0.288	0.163	1	0.012	0.296	-0.226	-0.079	-0.077
hsa-miR-874	0.062	0.017	0.012	1	-0.057	0.160	0.194	0.207
hsa-miR-454*	0.392	0.244	0.296	-0.057	1	-0.335	-0.328	0.076
hsa-miR-520c-3p	0.051	0.165	-0.226	0.160	-0.335	1	0.403	-0.149
hsa-miR-573	-0.067	-0.067	-0.079	0.194	-0.328	0.403	1	-0.171
hsa-miR-767-5p	-0.057	0.245	-0.077	0.207	0.076	-0.149	-0.171	1