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Expression quantitative trait loci (eQTLs) in microRNA genes are enriched for schizophrenia and bipolar disorder association signals

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Abstract

Background—Schizophrenia (SZ) and bipolar disorder (BD) have substantial negative impact on the quality of human life. Both, microRNA (miRNA) expression profiling in SZ and BD postmortem brains [and genome-wide association studies (GWAS)] have implicated miRNAs in disease etiology. Here, we aim to determine whether significant GWAS signals observed in the Psychiatric Genetic Consortium (PGC) are enriched for miRNAs.

Method—A two-stage approach was used to determine whether association signals from PGC affect miRNAs: (i) statistical assessment of enrichment using a Simes test and sum of squares test (SST) and (ii) biological evidence that quantitative trait loci (eQTL) mapping to known miRNA genes affect their expression in an independent sample of 78 postmortem brains from the Stanley Medical Research Institute.

Results—A total of 2567 independent single nucleotide polymorphisms (SNPs) ($R^2 > 0.8$) were mapped locally, within 1 Mb, to all known miRNAs (miRBase v. 21). We show robust enrichment for SZ- and BD-related SNPs with miRNAs using Simes (SZ: $p \leq 0.0023$, BD: $p \leq 0.038$), which remained significant after adjusting for background inflation in SZ (empirical $p = 0.018$) and approached significance in BD (empirical $p = 0.07$). At a false discovery rate of 10%, we identified a total of 32 eQTLs to influence miRNA expression; 11 of these overlapped with BD.

Conclusions—Our approach of integrating PGC findings with eQTL results can be used to generate specific hypotheses regarding the role of miRNAs in SZ and BD.

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Supplementary material

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Declaration of Interest

None.

Keywords

Bipolar disorder; eQTLs; GWAS; miRNAs; schizophrenia

Introduction

Schizophrenia (SZ) and bipolar disorder (BD) are debilitating psychiatric disorders that affect approximately 1% of the population worldwide (McGrath *et al.* 2008). Since 2007, when differential expression of microRNAs (miRNAs) in postmortem brain tissue from psychiatric patients and normal controls was assessed (Perkins *et al.* 2007) for the first time, increasing evidence from follow-up expression studies (Beveridge *et al.* 2008; Kim *et al.* 2010; Santarelli *et al.* 2011; Miller *et al.* 2013) conducted in postmortem brain tissue of SZ and BD patients as well as recent genome-wide association studies (GWAS) (PGC, 2011; Ripke *et al.* 2013, 2014) have implicated miRNAs to play a role in SZ and BD etiology. miRNAs are small single-stranded RNA molecules that mainly downregulate protein-coding genes by binding to the gene's 3′ untranslated region (UTR) (Forero *et al.* 2010) through a variety of mechanisms, including mRNA cleavage and degradation, translational inhibition, and shortening of the mRNA poly tail (Filipowicz *et al.* 2008).

In 2013, the Psychiatric Genetic Consortium (PGC) published the largest GWAS to date with an initial discovery sample of 21 856 individuals and a replication sample of 29 839 individuals (Ripke *et al.* 2013). Their strongest finding was located in the primary transcript of hsa-miR-137 (miR-137). Other genes, predicted as targets of miR-137, were also noted as achieving genome-wide significance; the predicted relationship between miR-137 and these genes has since been experimentally verified (Kim *et al.* 2012; Kwon *et al.* 2013; Collins *et al.* 2014). A second PGC study, utilizing an even larger sample size than the first, identified over 108 genome-wide significant signals, many of which also mapped to additional miRNA genes with neurodevelopmental function such as hsa-miR-640, -135a, -4304 and let-7g (Ripke *et al.* 2014). To our knowledge, however, no one has attempted to link aberrant miRNA expression from postmortem studies to significant genetic association signals identified in the PGC GWAS. Expression quantitative trait loci (eQTL) analysis offers a relatively straightforward approach to this task by linking genetic variants with gene expression (Westra & Franke, 2014). Moreover, specific testable hypotheses can be drawn from the significant associations found between variants and molecular features as eQTLs were shown to be also tissue- and cell-specific and to exhibit conserved effect sizes across major population groups (Stranger *et al.* 2012). miRNA-linked eQTLs, in particular, have been shown to be associated with the expression of complex phenotypic traits (Nicolae *et al.* 2010; Gamazon *et al.* 2012). miRNA-linked eQTLs can impact on miRNA cellular function either directly through modification of the miRNA expression (Hansen *et al.* 2007; Feng *et al.* 2009; Xu *et al.* 2010) or indirectly by impacting on the miRNA-binding activity to its target transcripts (Nicolae *et al.* 2010; Gamazon *et al.* 2012). In this study, we attempt to generate specific hypotheses regarding the genetic signals observed in the latest PGC GWAS and their connection to miRNAs by employing a two-stage approach. First, we ask whether GWAS signals observed in the PGC dataset are enriched for single nucleotide polymorphisms (SNPs) in linkage disequilibrium (LD, $R^2 > 0.8$) with known miRNA

precursors. Second, in a 1 Mb region flanking those miRNAs for which enrichment was detected, we ask, whether any of those specific SNPs function as eQTLs for miRNA expression. Multiple authors have noted already the enrichment of eQTLs among top GWAS findings in a wide variety of disorders, including SZ, diabetes, and specific types of cancer (Nicolae *et al.* 2010; Richards *et al.* 2012; Bacanu *et al.* 2014; Jiang *et al.* 2014; Roussos *et al.* 2014; Zhang *et al.* 2014). In this study we test two hypotheses: (1) that PGC GWAS signals are enriched for miRNA-linked eQTLs and (2) that by identifying the relationship between these genetic factors, the miRNAs and their respective gene targets, we can identify gene pathways of functional relevance to the pathophysiology of SZ and BD. Aberrant miRNA expression, especially during neurodevelopment, can modulate the expression of target protein-coding genes, which being themselves linked to the pathophysiology of SZ and BD as originally shown by PGC (PGC, 2011) could offer a novel etiological mechanism of disease development.

Method

Detection of SNP enrichment

All autosomal tag SNPs (Plink v1.07, $R^2 = 0.8$) (Purcell *et al.* 2007) found locally, within a 1 Mb region flanking known miRNA precursor sequences ($N = 1881$, miRBase v21) (Kozomara & Griffiths-Jones, 2014) were extracted and tested for enrichment in association signals over the PGC GWAS background using the Simes and sum of squares (SST) tests (see Supplementary material). Additionally, in order to identify potential confounders that may influence the level of GWAS enrichment, we performed bioinformatic analysis of the selected tag SNPs in our dataset. SNPs were excluded from our list if they were found to be located in highly conserved regions, predicted to have detrimental protein effects (Adzhubei *et al.* 2013) or if they mapped to highly conserved transcription factor binding sites.

To determine whether the miRNA location affects the level of enrichment, miRNAs were categorized as intergenic and intragenic (Down & Hubbard, 2002). Intergenic miRNAs are located outside of the boundaries of protein-coding genes; intragenic miRNAs are located within the boundaries of an annotated protein-coding gene, including those found within the 5′- and 3′-UTRs, and in the exonic and intronic regions of the gene. Intergenic miRNAs have been noted to possess an independent source of transcription, whereas intragenic miRNAs are often thought to share promoter sequences with the protein-coding genes that host them (Gromak, 2012; Ramalingam *et al.* 2014). It is worth noting that knowledge of transcription start sites (TSS) for the intragenic miRNAs has been based on bioinformatic prediction and the full range of the predicted miRNA TSS have not yet been fully validated experimentally (Saini *et al.* 2007). Indeed, recent experimental work using chromatin-based assays and miRNA cloning into empty promoter sequences have suggested that some intragenic miRNAs may have TSS that are transcribed by polymerases II and III (Pol II, Pol III) independently of their host gene (Borchert *et al.* 2006; Monteys *et al.* 2010). However, the data are still inconclusive and therefore, we believe that to assess the level of SNP enrichment separately based on this distinction alone would be premature without a full-scale validation of the TSS of known miRNAs and would also lead to a substantial reduction on statistical power. We do, however, draw a distinction between eQTLs associated with

intergenic *v.* intragenic miRNAs in our pathway enrichment analysis. Our rationale for this distinction is based on the potential confounding effect of miRNA location on its host gene; it is possible an eQTL affecting the expression of intragenic miRNA to also affect the expression of its host protein-coding gene. The distance parameters employed for determining whether a SNP is a 'local' or a cis-eQTL are based on previous studies in which cis-eQTLs have been identified (Gamazon *et al.* 2012, 2013*a*, *b*).

Data integration and genotype processing

All available genotypes were downloaded from the Stanley Online Genomics Database (Kim & Webster, 2010) and filtered with PLINK v1.02 (Supplementary material). Subjects having genotypes and miRNA expression values (Kim *et al.* 2010) were matched on the basis of gender, age, postmortem interval (PMI), brain pH, DSM-IV diagnosis, and gender. After applying a stringent quality control (QC) to the available genotype data, a total of 78 subjects (SZ = 27, BD = 29, controls = 22) were retained. Specific demographic information for these subjects can be found in online Supplementary Table S1. Imputation was performed on the original genotypes to make the GWAS panel used in the structural magnetic resonance imaging (sMRI) study more comparable to that used by the PGC (Supplementary material). A total of 309 531 SNPs in the sMRI GWAS was used to generate additional 332 271 genotypes meeting study criteria leading to a final count of 641 802 genotypes.

eQTL detection

eQTLs were detected using an additive model and accounting for the potential effect of age, brain pH, race, gender, DSM-IV classification, PMI and RNA integrity number (RIN) on miRNA expression. Those SNPs having missing data and/or a low minor allele frequency (MAF < 0.02) in the sMRI GWAS were not considered in the eQTL analysis. SNPs were coded as 0 (homozygous for the major allele), 1 (heterozygous) and 2 (homozygous for the minor allele) for evaluation in a linear regression model implemented by the R package Matrix EQTL (Shabalin, 2012); p values were corrected for multiple testing based on the number of SNPs within the 1 Mb region flanking of each individual miRNA that were tested in the enrichment stage at a false discovery rate (FDR) of 10%. We chose to focus exclusively on 'local' cis-eQTLs located within 1 Mb of the individual miRNA; by being closer to the gene of interest these have a greater chance to affect the overall level of expression than more distant (trans) eQTLs. A total of 2567 SNPs from the PGC dataset were evaluated in this manner.

Target prediction and pathway assessment

High-quality targets were predicted for all miRNAs for which a significant eQTL was identified (FDR < 0.1), using a consensus-based software approach in MiRWalk 2.0 (Dweep *et al.* 2011). MiRWalk 2.0 is a highly regarded software/database archive that documents predictions from 12 independent prediction algorithms, including DIANA-microTv4.0 (Maragkakis *et al.* 2011), DIANA-microT-CDS (Paraskevopoulou *et al.* 2013), miRanda-rel2010 (Betel *et al.* 2010), mirBridge (Tsang *et al.* 2010), miRDB4.0 (Wang and El Naqa, 2008), miRmap (Vejnar *et al.* 2013), miRNAMap (Hsu *et al.* 2008), doRiNA (Blin *et al.* 2015), PITA (Kertesz *et al.* 2007), PICTAR2 (Krek *et al.* 2005), RNAhybrid (Rehmsmeier *et*

al. 2004), and Targetscan (Grimson *et al.* 2007). MiRWalk 2.0 combines the information from these platforms to build miRNA target predictions in the promoter region, coding sequences, 5′- and 3′-UTRs. A consensus-based approach to target prediction has been recently suggested to be more useful since it minimizes the occurrence of false positives (Zhang & Verbeek, 2010; Pio *et al.* 2014; Yu *et al.* 2014). Targets for each miRNA were selected if they had a seed length ≥7 bases, were located with the gene's 3′-UTR and if the same binding site was predicted in ≥6 of the software systems that MiRWalk archives.

Using these targets, we analyzed the pathway enrichment using the human Wiki Pathways dataset, April 2014 release (Kelder *et al.* 2012). We first identified all genes targeted by miRNAs with significant cis-eQTLs which were then organized into gene pathways enriched for these miRNAs; second, the gene pathways were further assessed to identify pathways specifically enriched for the intragenic or intergenic miRNAs only. Pathway enrichment, at both stages, was determined using a hypergeometric test and correction for multiple testing was performed using methods outlined by Benjamini and Hochberg (Benjamini & Hochberg, 1995).

Results

Detection of SNP enrichment

Of the 2567 selected SNPs, four were shown by PolyPhen2 to be potentially impactful to mRNA/miRNA expression (score > 0.4). Furthermore, bioinformatic assessment of using SNPINFO (Xu & Taylor, 2009) indicated that the majority of SNPs had no clear predicted biological function related to the regulation of gene expression, with the exception of few being associated with conserved transcription factor binding sites (online Supplementary Table S2). We therefore believe that there was no other biological effect exerted by the SNPs on the level of enrichment other than through their relationship with the miRNAs.

Based on the Simes test, enrichment of miRNA SNPs associated with both SZ ($p = 0.0023$; Fig. 1*a*) and to a lesser extent with BD ($p = 0.038$, Fig. 1*b*) was detected. After applying the SST, which adjusts for the overall background enrichment of GWAS signals, the enrichment for SZ remained significant (empirical $p = 0.018$), while the enrichment for BD was suggestive (empirical $p = 0.07$). Detailed description of these two statistical approaches are provided in the online Supplementary material. The types of miRNAs that these SNPs tagged were roughly 2:1 (23 intragenic: nine intergenic miRNAs; Table 1), suggesting that, the source of the signal may have also originated from the mRNA genes that harbor them. However, despite the belief that intragenic miRNAs share promoters with their host mRNAs, recent studies suggest that this may not be entirely correct with some miRNAs such as hsa-miR-128-2 contain their own transcription regulatory elements (Ozsolak *et al.* 2008; Monteys *et al.* 2010). Therefore, to maximize our power, we have chosen to analyze both sets of SNPs as a single unit; however, as there might be distinct biological implications for the genes targeted by the intergenic *v.* intragenic miRNAs, thus we drew a clear distinction between these two classes of miRNAs when performing the pathway analysis.

eQTL mapping surrounding miRNAs

Within our enriched group of SNPs used in stage 1, we identified 116 SNPs to act as miRNA eQTLs at the nominal $p \leq 0.05$ of which 32 eQTLs remained significant following FDR correction at 10%. Of these 32 miRNA eQTLs, all were found to relate to SZ and 11 overlapped with BD. Our most significant SZ eQTL, rs3733047 ($p = 1.68 \times 10^{-6}$, $q = 0.001876$; Fig. 2*a*) was shown to affect the expression of hsa-miR-135a, which in a recent study was shown to be differentially expressed during the normal brain development (Moreau *et al.* 2013). Rs3733047 is located in a transcription factor binding site for GATA2 which regulates neuronal differentiation and has been found upregulated in the prefrontal cortex of SZ subjects (Miller *et al.* 2013). The second most significant SZ eQTL finding (rs6788142, $p = 2.16 \times 10^{-6}$, $q = 0.006754$; Fig. 2*b*) affects the expression of hsa-mir-138-1 (miR-138). miR-138 has been shown to decrease the amplitude of postsynaptic currents, negatively regulating spine size in mouse and rat studies (Olsen *et al.* 2009). The most significant BD eQTL, rs12119878 ($p = 0.001549$, $q = 0.010845$; Fig. 2*c*) was shown to affect the expression of hsa-miR-34a (miR-34a). An increased level of this miRNA was recently identified in the postmortem cerebellar tissue of BD patients (Bavamian *et al.* 2015); increased miR-34a expression in human iPSC-derived neuronal progenitor cells impacts neuronal differentiation and morphology. The second most significant BD related finding was rs10114192 ($p = 0.016858$, $q = 0.0118006$; Fig. 2*d*) that affected the expression of hsa-miR-32 and this miRNA was shown to be differentially expressed in post-mortem brains exclusively between BD patients and controls and not in SZ patients (Miller *et al.* 2013).

Other notable individual eQTLs include rs10993280 ($p = 0.00062592$, $q = 0.09213$) on chromosome 9 and rs7338471 ($p = 0.000116$, $q = 0.030872$) and rs4773657 ($p = 0.000437$, $q = 0.05369$) on chromosome 13. Rs10993280 is an eQTL for the hsa-miR-23b, -24-1, and -27b cluster on chromosome 9, while rs7338471 affected the expression of the hsa-miR-15a, and -16 cluster and rs4773657 is an eQTL for the hsa-19b-1, -17, -18a, -20a, and -92a cluster on chromosome 13.

Four eQTLs, rs4389575 ($p = 0.000622$, $q = 0.001036$), rs35930 ($p = 0.000676$, $q = 0.010133$), rs4661040 ($p = 0.005786$, $q = 0.098362$) and rs1422021 ($p = 0.000438$, $q = 0.065754$) were associated with the expression of four intragenic miRNAs; these miRNAs (hsa-mir-218-1, -449a, -9-1, and -103-1) have been experimentally shown to target their respective host genes (Wilfred *et al.* 2007; Bazzoni *et al.* 2009; Lize *et al.* 2010; Fish *et al.* 2011). A list of all 32 eQTLs affecting the expression of their respective miRNAs is given in Table 1. To further ensure the validity of our results as true signals, we compared our eQTLs against the Brain eQTL Almanac (Ramasamy *et al.* 2014); we found 82.1% of our eQTLs to be also reported in this database.

Of the 632 human pathways currently listed on WikiPathways, 588 showed significant enrichment of the genes predicted to be targets for the 32 miRNAs ($q < 0.01$). The pathways that demonstrated significant enrichment for intergenic miRNAs involved: (1) apoptosis, ($p = 2.00 \times 10^{-8}$, $q = 1.30 \times 10^{-6}$), (2) B cell receptor signaling pathway ($p = 8.29 \times 10^{-7}$, $q = 5.14 \times 10^{-5}$), (3) calcium signaling in cardiac cells ($p = 2.92 \times 10^{-8}$, $q = 1.90 \times 10^{-6}$), (4) G protein signaling ($p = 1.68 \times 10^{-6}$, $q = 1.02 \times 10^{-4}$), (5) insulin signaling ($p = 1.41 \times 10^{-4}$, q

= 5.76×10^{-3}), and (6) transforming growth factor beta (TGF-β) signaling ($p = 2.26 \times 10^{-4}$, $q = 7.70 \times 10^{-3}$). The pathways that demonstrated significant enrichment for intragenic miRNAs involved: (1) B cell receptor signaling ($p = 3.76 \times 10^{-8}$, $q = 2.22 \times 10^{-6}$), (2) DNA damage dependent ataxia telangiectasia, mutated (ATM) only ($p = 3.44 \times 10^{-7}$, $q = 1.96 \times 10^{-5}$), (3) insulin signaling ($p = 1.07 \times 10^{-9}$, $q = 6.29 \times 10^{-8}$) and (4) epidermal growth factor (EGF)/epidermal growth factor receptor (EGFR) signaling ($p = 1.63 \times 10^{-11}$, $q = 9.11 \times 10^{-10}$). Table 2 provides information describing all of the significantly enriched pathways.

Discussion

In this paper, we present results from a two-stage study that attempts to determine whether SNPs located in close proximity to miRNAs could be enriched for a SZ and BD association signal and whether these SNPs could function as eQTLs for the miRNAs to which they are co-localized. Our approach used the enrichment we detected in the PGC results as guidance for further fine mapping of the same variants in an eQTL analysis of postmortem brain samples from SZ and BD subjects. Our goal with the eQTL analysis in this study is to generate specific testable hypotheses that address the relationship between the observed GWAS signals and the miRNAs location, suggesting the potential impact this relationship might have on the targeted mRNA gene pathways.

Among these eQTLs, we speculate about two potential pathways by which a GWAS variant can interact with a miRNA in SZ and/or BD (pictured in Fig. 3). We show that SZ and BD significant SNPs could directly influence miRNA expression; however, the manner in which the SNP interacts with the miRNA might differ depending on the miRNA's origin. miRNAs in the intergenic category might be more strongly affected by an eQTL that would result in detectable levels of differential expression in postmortem expression studies because of the absence of other involved factors mediating this relationship. Of the miRNAs that fell into this group, it is important to note that hsa-miR-132, -34a, -124, -206 and -328 have been reported previously by other researchers to be associated with SZ/BD (Guo *et al.* 2010; Kim *et al.* 2010; Lai *et al.* 2011; Miller *et al.* 2013; Sun *et al.* 2015) in either postmortem expression studies or through genetic association. Furthermore, miRNAs within this class have been reported to be highly expressed in the brain, with miR-124 and -138 both being neurally specific and responsible for the differentiation of neural stem cells into neurons (Banerjee *et al.* 2009; Akerblom *et al.* 2012; Petri *et al.* 2014). Finally, hsa-miR-34a and -206, have also been noted to be responsive to mood stabilizers such as lithium and sodium valproate that provide support for their roles in SZ and BD (Hunsberger *et al.* 2013; Wang *et al.* 2014). The significantly enriched gene pathways unique to this group of miRNAs include the G protein signaling pathway and TGF-β signaling. Both of these pathways have been implicated in SZ and BD through risk genes in association studies, namely the G-protein-coupled receptors (GPCRs) (Funk *et al.* 2014) and ZNF804 (Umeda-Yano *et al.* 2013; Pietersen *et al.* 2014). GPCRs represent nearly half of the therapeutic drug targets in neuropsychiatric research today (Bridges & Lindsley, 2008). Both, hsa-miR-328 and some of its gene targets belonging to the TGF-β signaling pathway were also recently shown to be differentially expressed in laser-captured pyramidal neurons found in SZ subjects (Pietersen *et al.* 2014). Other enriched pathways of miRNAs in the intergenic class are involved in immune function and the regulation of signaling through calcium channels; both of these

pathways have been implicated in SZ and BD (Hinze-Selch, 2002; de Baumont *et al.* 2015) with respect to etiology and treatment course.

The second way in which SZ and BD significant SNPs could influence the expression of a miRNA is by affecting both the miRNA and its host gene in a regulatory loop, although it is unclear at present whether the effect of the SNP within the loop is equally shared between the miRNA and the host or not. The potential for regulatory loops involving miRNAs is nonetheless quite interesting with respect to SZ and BD disease etiology (Guo *et al.* 2010; Li *et al.* 2014; Shenoy & Blelloch, 2014). Several types of regulatory loops have been described; a feed forward loop (FFL), for instance, involves a transcription factor (TF) that binds to a cis-regulatory element upstream of the promoter for a miRNA and its mRNA target. In 'coherent' FFLs where the TF activates the miRNA that represses the mRNA gene, the controlling element in the loop is the TF and the relationship between all three elements is relatively balanced. In 'incoherent' FFLs, however, the TF activates both the miRNA and the mRNA; the relationship becomes more difficult to parse out because the action of the miRNA compensates against the activity of the TF on the mRNA gene. Several regulatory loops involving miRNAs have been described in connection to brain development and neuronal differentiation (Zhao *et al.* 2009; Sun *et al.* 2011; Liu *et al.* 2013). In this study, we found eQTLs for four miRNAs (hsa-miR-218-1, -449a, -9-3, and -103-1) for which there was experimental validation of the relationship between the host and the miRNA gene and instances of FFLs involving these same miRNAs (Wilfred *et al.* 2007; Bazzoni *et al.* 2009; Lize *et al.* 2010; Fish *et al.* 2011). We believe that with additional experimental validation of the relationship between the miRNAs in this class and their predicted targets, we will see additional examples of FFLs.

The enriched pathways targeted by the intragenic miRNA group were insulin signaling, B cell receptor signaling, DNA damage response only ATM dependent, and EGF/EGFR signaling. Two of these pathways, involving insulin signaling and B cell receptor signaling, overlapped with the intergenic group of miRNAs due to sequence similarities in hsa-miR-449a and -34a. The other enriched pathways that were unique to the intragenic miRNAs were the DNA damage response only ATM dependent and the EGF/EGFR signaling pathways. Of these, the EGF/EGFR signaling pathway has the most significant implications for neuropsychiatric disorders like SZ. The ligands for this pathway, in the ErbB families and neuregulin (NRG) families, are found in abundance in GABAergic and dopaminergic neurons and in glial cells. ErbB1 ligands, in particular, have been shown to negatively regulate GABAergic development in the neocortex and influence activity of glutamate receptor channels (Nagano *et al.* 2007). In addition, genetic and candidate gene studies have implicated both ErbB1-4 and NRG1-6 in SZ (Barnes *et al.* 2012; Deng *et al.* 2013; Nawaz *et al.* 2014).

By integrating eQTL analysis with the association signal seen in the PGCGWAS dataset, we can offer general hypotheses about the role of miRNAs in SZ and BD. First, due in large part to their location and the lack of other apparent factors directly mediating expression, the influence of intergenic miRNAs may be more readily apparent and directed in SZ/BD subjects than in intragenic miRNAs. The influence of intragenic miRNAs in SZ/BD might,

in turn, be easily missed if they participate in regulatory loops and focus is only given to the miRNA and not the other loop 'participants'.

We acknowledge several limitations to this study. First, there are significant challenges posed by working with postmortem brain samples that may lead to inconsistencies in study replication and reduced power (McCullumsmith *et al.* 2014). Factors such as age at death, PMI, brain PH, and prior exposure to specific medicines are potential confounders that, even when attempted to adjust for, may still confound the results from different expression studies when these are compared. In addition, although in recent years the numbers of postmortem brain samples have been steadily increasing, the sample size of currently available postmortem brain studies is still small, thus reducing statistical power and replicability. Furthermore, the composition of postmortem brain samples is often a mixture of tissues and cell types making it difficult to ascertain with certainty the exact source of the finding. The use of postmortem brains, nevertheless, offers the best possible substrate for studying complex human behavior simultaneously on a genetic and molecular level and provides a way in which to generate specific testable hypotheses that animal models may not allow.

A second perceived limitation of our study is that we deliberately chose not to distinguish the level of SZ and/or BD association enrichment between intergenic and intragenic miRNAs. Instead, we chose to look at the differences between the miRNA groups in terms of the predicted targets of each group and their respective pathways. Indeed, while we cannot unequivocally state that the level of enrichment did not result from the influence of the protein coding genes that hosted the intragenic miRNAs, we would still suggest that the effect is nonetheless present and inextricably linked to miRNAs. What our study does suggest is that certain classes of miRNAs may be more directly connected to genetic factors and that these may be the ones for whom neuronal cell signaling and fate might be a key function. In that regard, our study provides a successful approach to breach the gap between purely genetic (associations) and molecular (expression) studies conducted in neuropsychiatric disorders such as SZ and BD.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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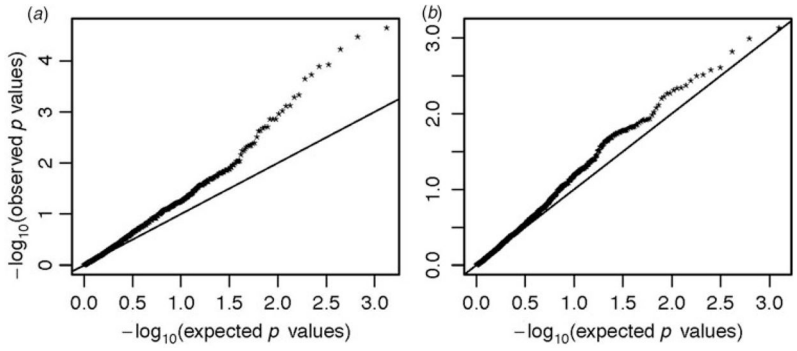

Fig. 1.

Quantile–quantile (QQ) plot of the genome-wide association studies signals enrichment among the miRNA expression quantitative trait loci in schizophrenia (SZ) and bipolar disorder (BD). The expected $-\log^{10} p$ values under the null hypothesis are represented on the x axis, while the observed values are represented on the y axis. (*a*) Shows the enrichment for SZ single nucleotide polymorphisms (SNPs) and (*b*) for BD SNPs.

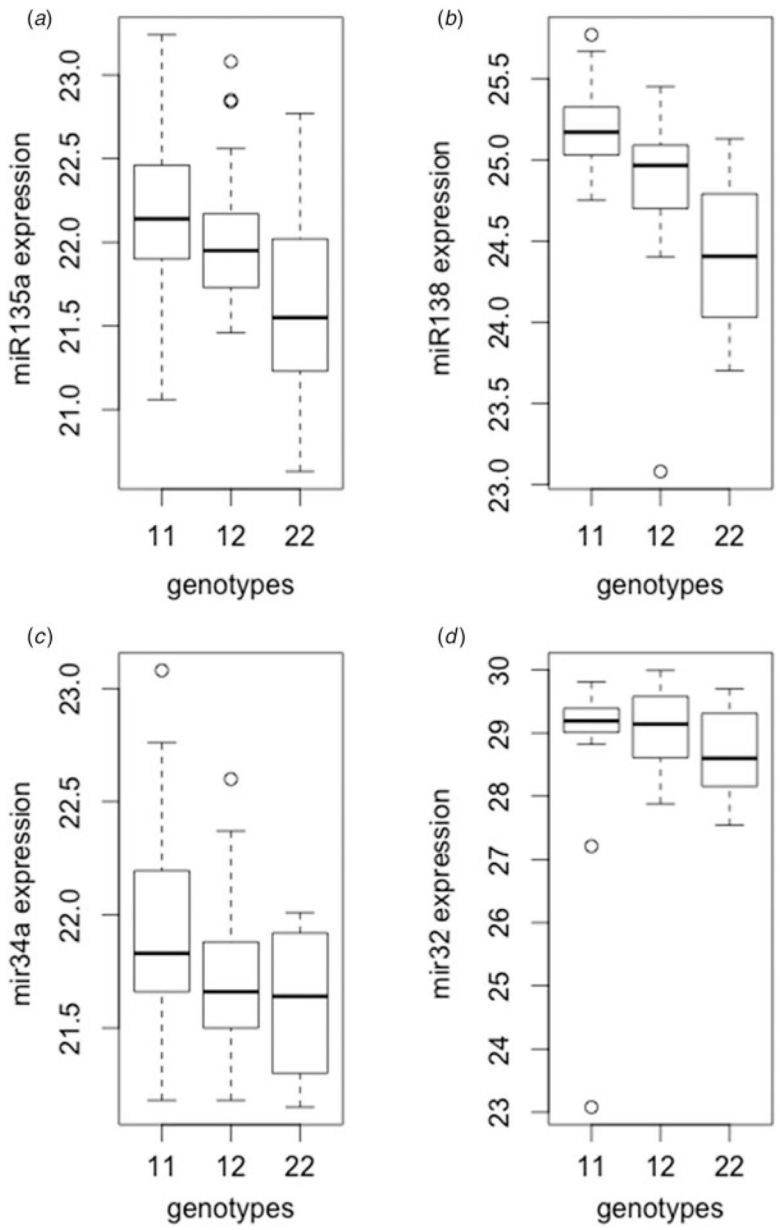

Fig. 2.

Boxplots showing the relationship between single nucleotide polymorphism genotype and miRNA expression for the two most significant schizophrenia (SZ) and bipolar disorder (BD) expression quantitative trait loci (eQTLs). (*a*) and (*b*) show SZ-related eQTLs, while (*c*) and (*d*) show BD-related eQTLs.

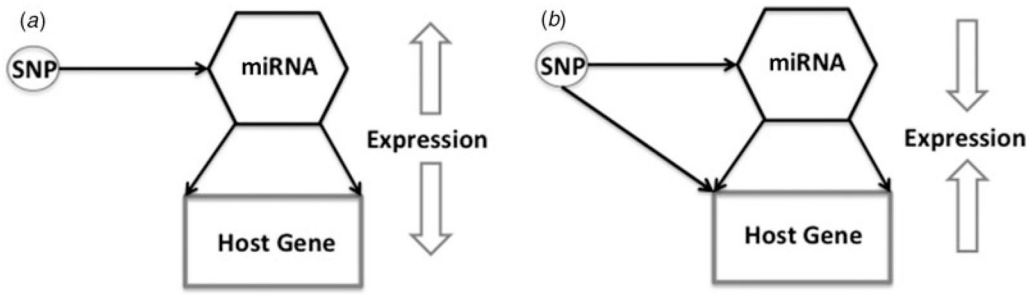

Fig. 3.

A schematic diagram depicting the hypothesized relationship between expression quantitative trait loci, miRNAs, and mRNA genes. (*a*) Depiction of 'coherent' feed forward loop (FFL) and (*b*) depiction of 'incoherent' FFL. See text for more details. SNP, single nucleotide polymorphism.

Table 1

A master table showing pertinent information to the detection of 32 eQTLs and the miRNAs to which they are linked

Disorder	miRNA	miRNA origin	Chromosome	miRNA base pair location	eQTL	eQTL base pair location	Alleles	Global MAF	eQTL *p* value	eQTL *q* value
SZ/BD	hsa-miR-34a	Intergenic	1	9151668-9151777	rs12119878	9158662	G/T	0.1967	1.55×10^{-4}	1.08×10^{-2}
SZ	hsa-miR-138	Intergenic	3	44114212-44114310	rs6788142	44128954	A/G	0.1899	2.16×10^{-6}	6.75×10^{-3}
SZ/BD	hsa-mir-206	Intergenic	6	52144349-52144434	rs6921008	52610560	C/T	0.2274	1.88×10^{-4}	8.46×10^{-2}
SZ/BD	hsa-mir-124-1	Intergenic	8	9903388-9903472	rs7461939	9714111	A/G	0.4407	1.07×10^{-3}	2.90×10^{-2}
SZ	hsa-mir-1251	Intergenic	12	97491909-97491978	rs4762481	98310417	A/G	0.3349	3.64×10^{-4}	7.28×10^{-2}
SZ	hsa-mir-494	Intergenic	14	101029634-101029714	rs9324038	101408012	A/G	0.2015	2.55×10^{-4}	7.53×10^{-3}
SZ	hsa-mir-138-2	Intergenic	16	56858518-56858601	rs1566439	56990750	A/G	0.3806	3.38×10^{-3}	8.45×10^{-2}
SZ/BD	hsa-mir-328	Intergenic	16	67202321-67202395	rs28171	66537518	A/G	0.0519	2.92×10^{-4}	7.00×10^{-2}
SZ/BD	hsa-mir-132-3p	Intergenic	17	2049908-2050008	rs17834974	2742577	A/G	0.2726	7.07×10^{-4}	8.84×10^{-2}
SZ	hsa-mir-9-1	Intragenic	1	156420341-156420429	rs4661040	156399474	A/G	0.2849	8.28×10^{-4}	2.24×10^{-2}
SZ/BD	hsa-mir-205	Intragenic	1	209432133-209432242	rs11577858	209404159	A/G	0.3478	5.34×10^{-4}	6.68×10^{-2}
SZ	hsa-mir-664	Intragenic	1	220200538-220200619	rs6669360	219474597	A/T	0.3636	1.71×10^{-3}	5.98×10^{-2}
SZ	hsa-mir-153	Intragenic	2	219294111-219294200	rs1425029	219729817	A/G	0.3736	1.43×10^{-3}	5.01×10^{-2}
SZ	hsa-mir-217	Intragenic	2	55982967-55983076	rs7604029	56042395	C/T	0.3231	7.96×10^{-4}	7.16×10^{-2}
SZ	hsa-miR-135a	Intragenic	3	52294219-52294308	rs3733047	52837913	A/G	0.2452	1.68×10^{-6}	1.88×10^{-3}
SZ	hsa-mir-218-1-3p	Intragenic	4	20528275-20528384	rs4389575	21020885	A/G	0.4599	6.22×10^{-4}	1.04×10^{-2}
SZ	hsa-mir-874	Intragenic	5	137647572-137647649	rs10060914	137497671	A/G	0.3766	1.74×10^{-4}	1.56×10^{-2}
SZ	hsa-mir-103-1	Intragenic	5	168560896-168560973	rs1422021	169357195	A/G	0.2107	4.38×10^{-4}	6.58×10^{-2}
SZ	hsa-mir-449a	Intragenic	5	55170532-55170622	rs35930	54285894	A/G	0.2752	6.76×10^{-4}	1.01×10^{-2}
SZ	hsa-mir-597	Intragenic	8	9741672-9741768	rs735449	9245937	G/T	0.2388	8.24×10^{-4}	2.88×10^{-2}
SZ/BD	hsa-mir-32	Intragenic	9	109046229-109046298	rs10114192	108859784	C/T	0.497	1.69×10^{-2}	1.18×10^{-2}
SZ	hsa-mir-601	Intragenic	9	123402525-123402603	rs10739620	122840801	C/G	0.1378	2.12×10^{-4}	7.42×10^{-3}
SZ	hsa-mir-101	Intragenic	9	4850297-4850375	rs10974788	4795595	G/T	0.1819	1.69×10^{-4}	1.18×10^{-2}
SZ	hsa-mir-7-1-3p	Intragenic	9	83969748-83969857	rs1502683	82984665	C/T	0.4323	2.69×10^{-3}	9.42×10^{-2}
SZ/BD	hsa-mir-27b	Intragenic	9	95085445-95085541	rs10993280	94647489	C/T	0.4782	6.25×10^{-4}	9.21×10^{-2}
SZ/BD	hsa-mir-148b	Intragenic	12	54337216-54337314	rs2306851	54854422	C/T	0.1372	7.33×10^{-3}	9.53×10^{-2}

Psychol Med. Author manuscript; available in PMC 2016 April 26.

Disorder	miRNA	miRNA origin	Chromosome	miRNA base pair location	eQTL	eQTL base pair location	Alleles	Global MAF	eQTL *p* value	eQTL *q* value
SZ/BD	hsa-mir-16-1-3p	Intragenic	13	50048973-50049061	rs7338471	49406928	G/T	0.3548	1.16×10^{-4}	3.09×10^{-2}
SZ	hsa-mir-19b-1-3p	Intragenic	13	91351192-91351278	rs4773657	91963767	C/T	0.4692	4.37×10^{-4}	5.37×10^{-2}
SZ/BD	hsa-mir-190a	Intragenic	15	62823957-62824041	rs12438131	63465635	C/G	0.4998	5.55×10^{-3}	1.39×10^{-2}
SZ	hsa-mir-484	Intragenic	16	15643294-15643372	rs6498573	15785516	C/T	0.2236	3.08×10^{-3}	8.33×10^{-2}
SZ	hsa-mir-744	Intragenic	17	12081899-12081996	rs1468501	12018627	C/T	0.1965	1.19×10^{-3}	1.07×10^{-2}
SZ	hsa-mir-195	Intragenic	17	7017615-7017701	rs11655246	6124519	C/T	0.345	3.65×10^{-5}	1.61×10^{-2}

eQTL, Expression quantitative trait loci; miRNA, microRNA; MAF, minor allele frequency; SZ, schizophrenia; BD, bipolar disorder.

Table 2

A summary table showing the enriched pathways predicted to be targeted by the 32 miRNAs

miRNA type	miRNAs showing significant levels of target enirchment	Wiki pathway name	Number of genes in a given pathway	Average no. of predicted targets in pathway	Single most enriched miRNA	*p* value	*q* value
Intergenic	124-1, −1251, −132-3p, −138-1,−138-2, −206, −328, −34a	Apoptosis	84	55	mir34a	2.00×10^{-8}	1.30×10^{-6}
Intergenic	124-1, 1251, 132-3p, 138-1, 138-2, 206, 328, 34a	B cell receptor signaling pathway	95	60	mir34a	8.29×10^{-7}	5.14×10^{-5}
Intergenic	124-1,−1251, −132-3p, −138-1, −138-2, −206, −328, −34a	Calcium regulation in the cardiac cell	150	90	mir34a	2.92×10^{-8}	1.90×10^{-6}
Intergenic	124-1, 1251, 132-3p, 138-1, 138-2, 206, 328, 34a	G protein signaling pathways	93	57	mir34a	1.68×10^{-6}	1.02×10^{-4}
Intergenic	124-1, 1251, 132-3p, 138-1, 138-2, 206, 328, 34a	Insulin signaling	164	96	mir34a	1.41×10^{-4}	5.76×10^{-3}
Intergenic	124-1 −132-3p, −138-1, −138-2,−206, −328, −34a, −494	TGF-β signaling pathway 1	120	68	mir138-2	2.26×10^{-4}	7.70×10^{-3}
Intragenic	101, 103a, 135a, 16-1, 205, 218, 27b, 449a, 601, 664a, 7-1	B cell receptor signaling pathway	95	65	mir744	3.76×10^{-8}	2.22×10^{-6}
Intragenic	101-3p, 103a-3p, 135a-5p,153-3p, 16-1-3p, 195-5p, 205-5p, 218-1, 27b −3p, 449a. 601, 744-5p,	DNA damage response only ATM dependent	84	57	mir744	3.44×10^{-7}	1.96×10^{-5}
Intragenic	101-3p, 103a-3p, 135a-3p. 153-3p, 16-1-3p, 190a-5p, 195-5p, 205-5p, 217, 218-1, 27b-3p, 449a, 597-3p, 664a-5p, 7-1-3p, 744-5p, 874-5p	Insulin signaling	164	96	mir205-5p	1.07×10^{-9}	6.29×10^{-8}
Intragenic	101-3p, 103a-3p, 135a-5p, 153-3p, 195-5p, 205-5p, 217, 218-1, 27b-3p, 449a, 601, 744, 9-3	EGF/EGFR signaling pathway	145	93	mir27b-3p	1.63×10^{-11}	9.11×10^{-10}

miRNA, MicroRNA; TGF-β, transforming growth factor beta; ATM, ataxia telangiectasia, mutated; EGFR, epidermal growth factor receptor.

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