Original Investigation

Analyzing the Role of MicroRNAs in Schizophrenia in the Context of Common Genetic Risk Variants

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IMPORTANCE The recent implication of 108 genomic loci in schizophrenia marked a great advancement in our understanding of the disease. Against the background of its polygenic nature there is a necessity to identify how schizophrenia risk genes interplay. As regulators of gene expression, microRNAs (miRNAs) have repeatedly been implicated in schizophrenia etiology. It is therefore of interest to establish their role in the regulation of schizophrenia risk genes in disease-relevant biological processes.

OBJECTIVE To examine the role of miRNAs in schizophrenia in the context of disease-associated genetic variation.

DESIGN, SETTING, AND PARTICIPANTS The basis of this study was summary statistics from the largest schizophrenia genome-wide association study meta-analysis to date (83 550 individuals in a meta-analysis of 52 genome-wide association studies) completed in 2014 along with publicly available data for predicted miRNA targets. We examined whether schizophrenia risk genes were more likely to be regulated by miRNA. Further, we used gene set analyses to identify miRNAs that are regulators of schizophrenia risk genes.

MAIN OUTCOMES AND MEASURES Results from association tests for miRNA targetomes and related analyses.

RESULTS In line with previous studies, we found that similar to other complex traits, schizophrenia risk genes were more likely to be regulated by miRNAs ($P < 2 \times 10^{-16}$). Further, the gene set analyses revealed several miRNAs regulating schizophrenia risk genes, with the strongest enrichment for targets of miR-9-5p ($P = .0056$ for enrichment among the top 1% most-associated single-nucleotide polymorphisms, corrected for multiple testing). It is further of note that *MIR9-2* is located in a genomic region showing strong evidence for association with schizophrenia ($P = 7.1 \times 10^{-8}$). The second and third strongest gene set signals were seen for the targets of miR-485-5p and miR-137, respectively.

CONCLUSIONS AND RELEVANCE This study provides evidence for a role of miR-9-5p in the etiology of schizophrenia. Its implication is of particular interest as the functions of this neurodevelopmental miRNA tie in with established disease biology: it has a regulatory loop with the fragile X mental retardation homologue *FXR1* and regulates dopamine D_2 receptor density.

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Schizophrenia is a common psychiatric disorder with considerable morbidity,[1] high heritability,[2] and extensive genetic heterogeneity.[3] Herein, we examine the disease from the perspective of a potential influence of microRNAs (miRNAs), which are approximately 22-nucleotide-long endogenous RNA molecules that regulate gene expression posttranscriptionally by pairing with the RNA-induced silencing complex, subsequently binding messenger RNA (mRNA), and inducing translational repression and/or mRNA degradation. Computer models have been widely used to predict such interactions as detailed in eAppendix 2 in the Supplement.

Accumulating evidence implicates miRNAs with schizophrenia: miRNAs are known to play important roles in brain development[4]; miRNAs are found differentially expressed in postmortem brains of patients with schizophrenia[5]; and miRNAs and their targets are found enriched in risk loci from genetic studies at the level of copy number variations (CNVs) as well as common and rare variations.[6-9] Additionally, much of the signal in genome-wide association studies (GWASs) of schizophrenia is believed to come from variants altering gene expression,[10,11] thus putting miRNAs as regulators of gene expression into the spotlight.

In this study, the role of miRNAs in the etiology of schizophrenia is analyzed at 3 levels using the following approaches: (1) by assessing whether schizophrenia risk genes overall are more likely to be regulated by miRNAs; (2) by gene set analyses to find conserved miRNAs that are regulators of schizophrenia risk genes; and (3) by targeted gene set analyses to systematically characterize the importance of miRNAs in previously identified risk loci from GWASs and CNVs.

Methods

The basis for the analyses in this article (if not stated otherwise) is summary statistics from the most recent schizophrenia GWAS meta-analysis conducted by the Schizophrenia Working Group of the Psychiatric Genomics Consortium (PGC2).[12] Institutional review board approval was obtained for PGC2 where required and was described in detail previously.[12] As this study was a secondary analysis of deidentified data, neither informed consent nor institutional review board approval was required. For the purpose of our analyses, only autosomal results were used, and tests excluded the broader major histocompatibility complex (MHC) region (chr6:25M-35M).

Regulation of Schizophrenia Risk Genes by miRNAs

In a first step, we aimed to gain a global measure of the magnitude to which schizophrenia risk genes are regulated by miRNA. We therefore examined whether the degree to which a gene was regulated by miRNA correlated with the gene's association with schizophrenia by applying a linear model of log-transformed gene P values with gene and 3′ untranslated region lengths included as covariates with miRNA target predictions from TargetScan[13] (**Figure 1**A and eAppendix 2 in the Supplement). To study the specificity of our findings, we repeated the analyses with summary statis-

tics from well-powered GWASs for age at menarche, Crohn disease, and height.[14-16]

Gene Set Enrichment Analyses of All Conserved miRNAs

A flowchart of the gene set analyses in this article is presented in Figure 1B. In the following, we provide information on specific aspects of our analyses (see eAppendix 2 in the Supplement for more information).

miRNAs and Their Targets

Names and genomic locations of miRNAs and stem-loop structures were taken from mirBase 20.[17] Based on characteristics of RNA-sequencing experiments, some miRNAs are classified as *high confidence* and are considered to have a high probability of representing a bona fide miRNA. For miRNA target sites, TargetScan 6.2 conserved target sites of conserved miRNA families[13] were used unless otherwise stated. Because of its reliance on conservation and the requirement for miRNAs to have a seed site, the predicted targets of this algorithm have a higher chance of being functionally important. For the TargetScan miRNA families, only names of human miRNAs in each family are listed and only gene sets with more than 50 genes were considered. National Center for Biotechnology Information protein coding genes and their corresponding hg19 positions were used.

Statistical Approach

We used INRICH[18] for all gene set analyses. This method tests the overlap between genomic intervals associated with the trait of interest and predefined gene sets. Linkage disequilibrium, variable gene lengths, and variable single-nucleotide polymorphism (SNP) and gene density are taken into account and multiple testing is corrected using a bootstrapping approach. For our analyses, SNPs were filtered for a minor allele frequency of 1% or greater and info score of 0.8 or higher. The SNPs were clumped with PLINK 1.9 using all samples of European ancestry from the 1000 Genomes Project phase 1[19] with the following settings: for the index SNP, 3 significance thresholds were used, 1×10^{-5}, 3.420×10^{-4}, and 0.0110, with the latter 2 values corresponding to a threshold for the top 1% and top 5% of all SNPs outside the MHC. In all 3 cases, $r^2 = 0.6$ and a window of 500 kilobases (kb) were used, ie, the same parameters that were used to define the associated loci in PGC2.

Scoring the Results

When analyzing the results from INRICH, it was noted that sometimes the P value for the gene set would fluctuate when using different P value thresholds. Furthermore, INRICH gives

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Key Points

Question: What is the role of microRNAs in schizophrenia in the context of common genetic risk variants?

Findings: Several microRNAs regulate schizophrenia risk genes, with the strongest associations for miR-9-5p, miR-485-5p, and miR-137.

Meaning: There is evidence that miR-9-5p is itself a risk gene, and its function ties in with established disease biology.

Figure 1. Flowcharts of the Analyses
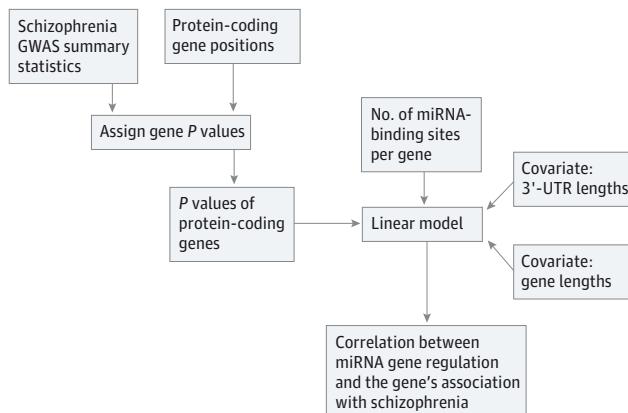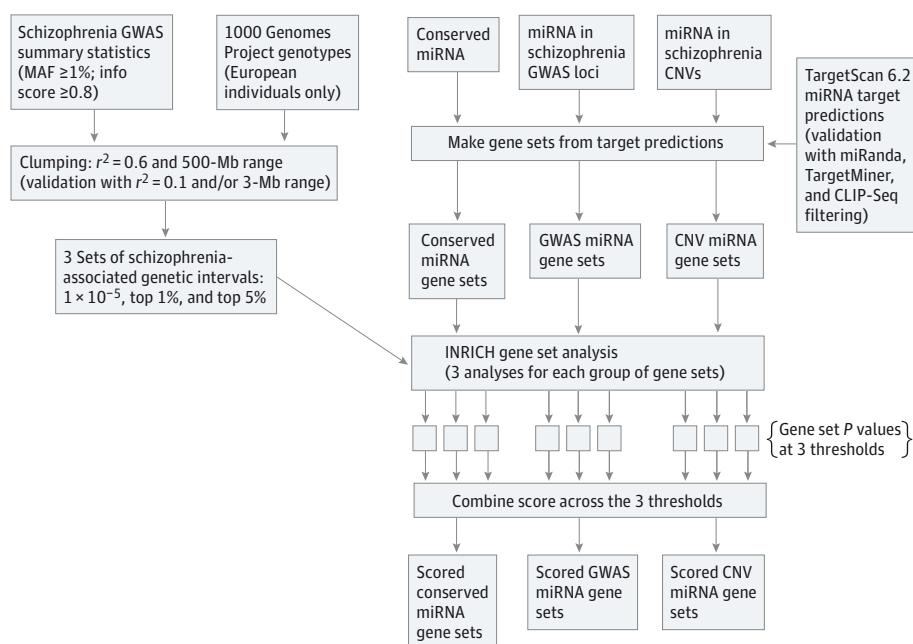
A, Flowchart of the linear model for assessing whether schizophrenia risk genes are more likely to be targeted by microRNAs (miRNAs). GWAS indicates genome-wide association study; UTR, untranslated region. B, Flowchart for gene set analyses of all conserved miRNAs and the targeted gene set analyses. CLIP-Seq indicates cross-linking immunoprecipitation followed by sequencing; CNV, copy number variation; MAF, minor allele frequency; and Mb, megabase. More information on the analytical strategies can be found in the text.

the same weight to all intervals regardless of how significantly associated they are. This is undesirable, as more significantly associated intervals are more likely to be true risk loci. To circumvent these limitations, a score was assigned to each gene set: $\Pi^3_{i=1}[1 - \log(P_i)]$, where P_i is the P value corrected for multiple testing of the gene sets based on the ith inclusion threshold and where log is the natural logarithm. This results in a score that weighs by strength of association and gives higher weight to gene sets that show association across all thresholds.

Characterization of Potential Confounders

First, we examined whether our top-scoring gene sets were simply those with the highest content of brain-expressed genes.

For each gene set, the number of brain-expressed genes was calculated based on information from the eGenetics/SANBI EST anatomical system data (Ensembl 75).[20] To study the specificity of our findings, we repeated our TargetScan-based analyses for our top miRNA gene sets in 3 unrelated traits[14-16] (see earlier). We also studied the effect of different clumping thresholds on our results through comparison of results from all possible combinations for r^2 choices of 0.1 and 0.6 and/or a window size of 500 kb and 3000 kb. Finally, we studied the effect of target prediction algorithms by using TargetScan predictions filtered with data from 58 AGO cross-linking immunoprecipitation experiments[21-23] and the 2 additional target prediction resources TargetMiner[24] and miRanda[25] (eAppendix 2 in the Supplement).

Table. Top 10 Conserved miRNA Gene Sets

TargetScan 6.2 miRNA Family	P Value at Different Thresholds[a]			Score	Genes, No.	Brain, %[b]
	1 × 10⁻⁵	Top 1%	Top 5%			
miR-9-5p	.0378[c]	.0056[d]	.0009[e]	212.3	1237	75
miR-485-5p	.1474	.1373	.0011[d]	68.3	379	73
miR-137	.0844	.0326[c]	.0319[c]	68.3	1144	77
miR-101-3p	.2358	.0135[d]	.0211[c]	63.0	803	78
miR-200bc-3p/429	>.99	.0050[d]	.0011[d]	49.5	1057	77
miR-7-5p	.4311	.0555	.0236[c]	34.0	444	73
miR-1/206/613	.2143	.3825	.0052[d]	31.1	787	76
miR-374ab-5p	.8970	.0075[d]	.0304[c]	29.4	678	71
miR-28-5p/708-5p/3139	.0716	.0380[c]	.4152	29.2	209	80
miR-34ac-5p/449b-5p/449a	.1951	.2136	.0793	23.7	655	78

Abbreviation: miRNA, microRNA.

[a] P values are corrected for multiple testing within each threshold for all 143 tested gene sets using INRICH's bootstrapping approach. A Bonferroni-corrected α level of .017 should be applied to correct for all tests performed in our analyses. Owing to correlations in the results for the different thresholds, a Bonferroni correction seems to be too conservative. The 3 different thresholds represent the different significance thresholds for the index single-nucleotide polymorphism used in clumping. The top 1% of single-nucleotide

polymorphisms have $P < 3.420 \times 10^{-4}$; the top 5% of single-nucleotide polymorphisms have $P < .0110$.

[b] Indicates the percentage of the test genes expressed in the brain.

[c] $P < .05$.

[d] $P < .017$.

[e] $P < .001$.

Follow-up of Findings

To expand on our findings in the gene set analysis, we used a framework of different approaches to characterize the relationship of our top miRNAs and their targets with schizophrenia. In brief, we checked for association of our top miRNAs in the PGC2 GWAS,[12] analyzed the overlaps in targets of different miRNAs, used the DAVID tools[26] for functional annotation of the miRNA targets, and used BrainSpan[27] to establish spatiotemporal expression patterns. We also used BrainSpan to identify coexpressed clusters of targeted genes, which we subsequently characterized; we examined their enrichment for common and rare variants using data from PGC2 and a recently published exome-sequencing study in schizophrenia,[28] respectively. Furthermore, we tested for excess in protein-protein interactions within each significantly associated cluster using data from TissueNet.[29] We also looked at differential expression in data from postmortem brains of patients with schizophrenia and controls[30] in an additional attempt to identify submodules of schizophrenia risk genes targeted by our top-ranking miRNAs. These analyses are further detailed in eAppendix 2 in the Supplement.

Targeted Gene Set Enrichment Analyses

In addition to our analyses of gene set enrichment of all conserved miRNAs, we used a targeted gene set analysis approach to further characterize recent findings from GWAS and CNV analyses.[12,31] Targetomes of miRNAs located in the 108 schizophrenia GWAS loci[12] from the PGC2 study were examined. These GWAS miRNAs were defined as miRNAs whose primary miRNA (pri-miRNA) genetic sequences overlapped with one of the GWAS loci. In addition to the targetomes of GWAS miRNA, we also analyzed targetomes of miRNA located in 10 schizophrenia-associated CNVs identified in a recent meta-analysis.[31] These CNV miRNAs were defined as those miRNAs whose pri-miRNA genetic sequence overlapped with

one of the 10 CNVs. All targeted gene set analyses were conducted as described for the analyses of gene set enrichment of all conserved miRNAs with a single exception: for the TargetScan-based analyses, all predicted targets regardless of conservation were used, as only a few of the identified miRNAs were conserved.

Results

Regulation of Schizophrenia Risk Genes by miRNAs

We found a negative correlation between the log(P value) of a protein-coding gene and its corresponding number of predicted miRNA sites ($\beta = -0.016$; $P < 2 \times 10^{-16}$), ie, genes with more predicted miRNA target sites showed on average a stronger association with schizophrenia. However, 2 of 3 additionally tested GWAS traits showed a similar tendency (eAppendix 2 in the Supplement). Considering just the 108 genome-wide–significant schizophrenia loci, protein-coding genes located in these have on average a 21% excess of predicted miRNA-binding sites compared with protein-coding genes in general.

Gene Set Enrichment Analyses of All Conserved miRNAs

Testing the targetomes of conserved miRNA, several schizophrenia-associated gene sets were found using the predictions of TargetScan (Table and eTable 1 in the Supplement). The 10 highest-scoring miRNA targetomes are illustrated as a Circos plot[32] in Figure 2, in a cluster plot in Figure 3, and further in eFigure 1 and eFigure 2 in the Supplement. Our top-ranking miRNA gene sets were not simply the largest gene sets or those with the highest number or fraction of brain-expressed genes (eTable 1 in the Supplement). Furthermore, our findings were largely consistent under alternative test conditions. This included additional analyses carried out with less

Figure 2. Circos Plot of the Top 10 Schizophrenia MicroRNA (miRNA) Gene Sets
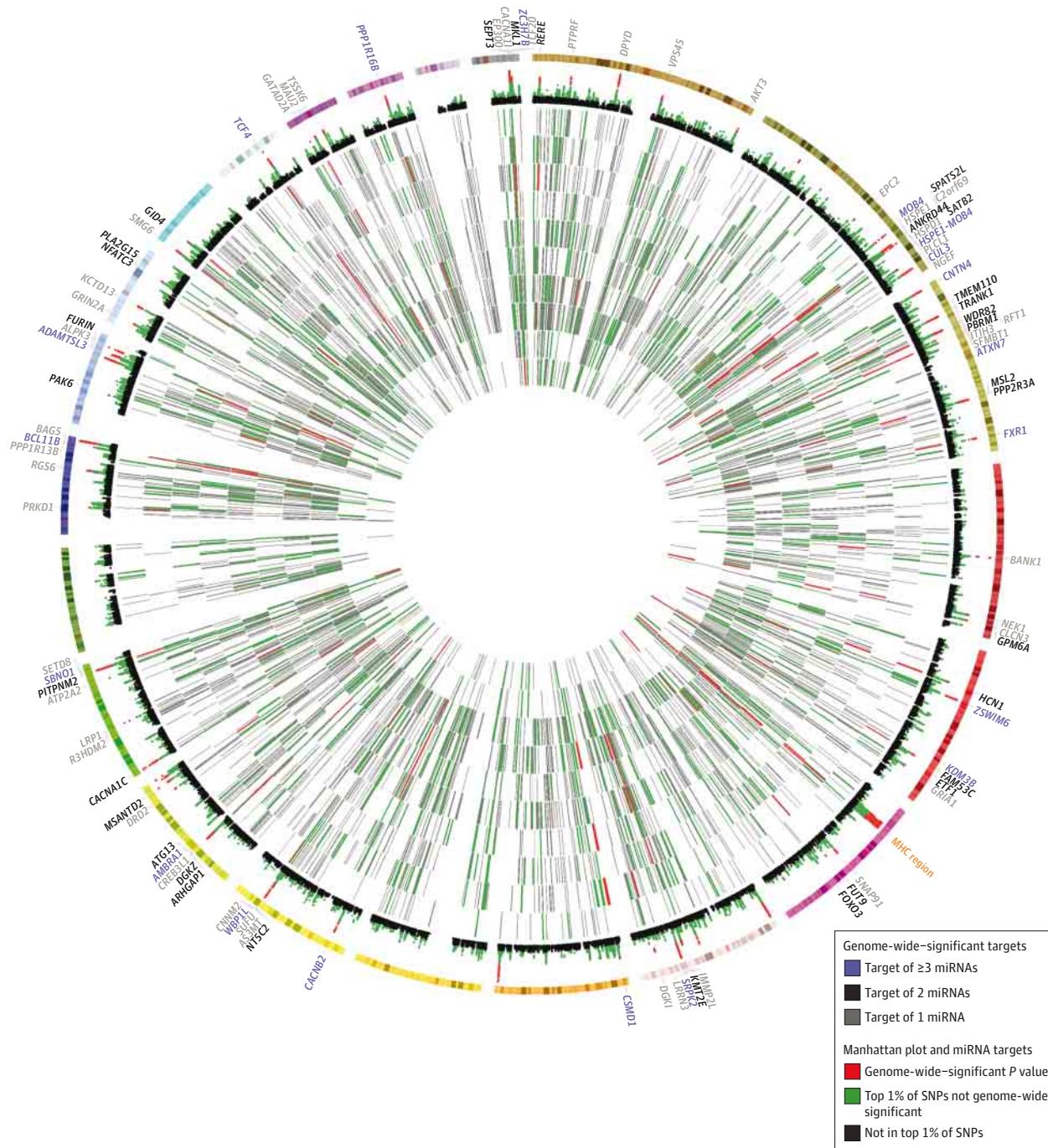
The innermost 10 tracks illustrate the targets of each miRNA. The targets are color coded based on their gene *P* values. The miRNAs were ordered by their correlational clustering. Peripherally to this, a Manhattan plot is shown (only single-nucleotide polymorphisms [SNPs] with *P* < .02 located in protein-coding genes are included). At the edge, the genome-wide–significant genes targeted by the top 10 miRNAs are shown. They are color coded based on the number of miRNAs in the top 10 list that target them. The major histocompatibility complex (MHC) region is included here for illustrative purposes but was not part of the gene set tests, and *P* values from the most recent schizophrenia genome-wide association study meta-analysis conducted by the Schizophrenia Working Group of the Psychiatric Genomics Consortium (PGC2)[12] are without replication. In eFigure 1 in the Supplement, a zoomed-in view of this region is presented.

strict thresholds for linkage disequilibrium and/or longer windows in clumping (eTable 2, eTable 3, and eTable 4 in the Supplement) and tests with 2 additional miRNA prediction algorithms (eTable 5 and eTable 6 in the Supplement). Filtering

Figure 3. Clustering of MicroRNAs (miRNAs) Based on the Jaccard Distance Between the Targets of Each miRNA

Height indicates the dissimilarity measure in the clustering; SNPs, single-nucleotide polymorphisms. In eFigure 2 in the Supplement, the clustering is repeated considering only the targets showing increasing degrees of association with schizophrenia.
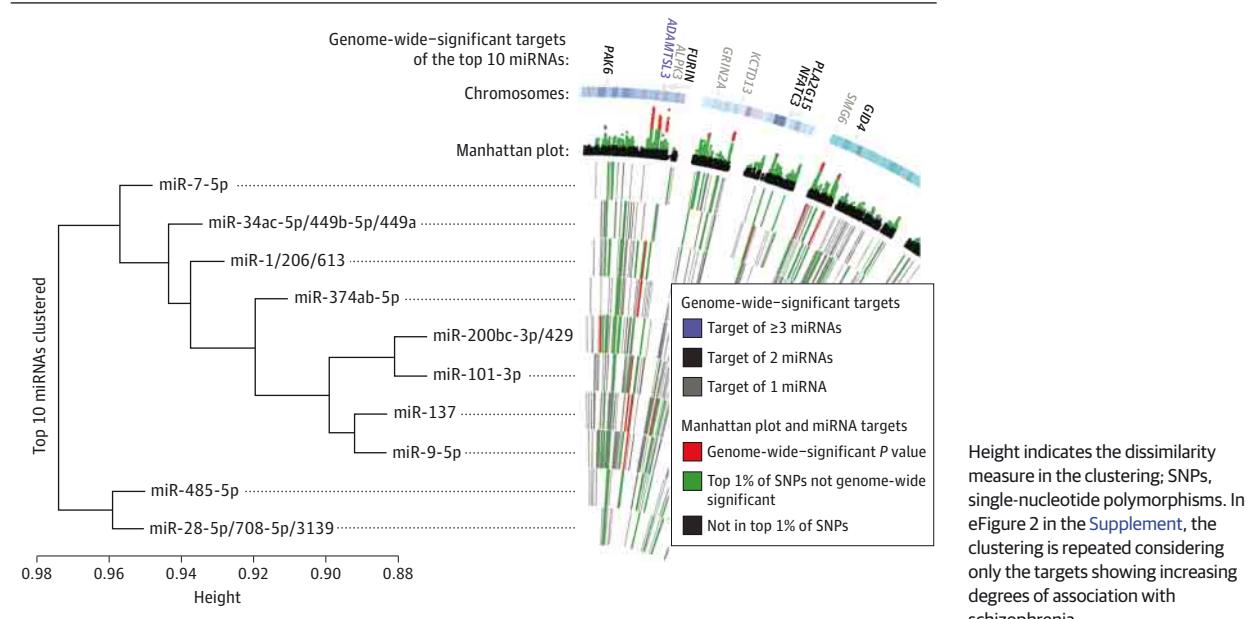
with cross-linking immunoprecipitation data, which on average removed 45% of TargetScan-predicted targets, was not found to be better than removing genes at random (eAppendix 2 and eTable 7 in the Supplement). Further, our top 10 miRNAs showed no evidence for association in well-powered studies of unrelated traits (eTable 8 in the Supplement). In a first attempt to further characterize our findings (using DAVID[26]), we found enrichment of genes targeted by 2 or more miRNAs in our top 10 in terms related to transcriptional regulation and neuronal development (eTable 9 and eTable 10 in the Supplement).

miR-9-5p and Related Follow-up Analyses

In our analysis of conserved miRNAs, the targetome of miR-9-5p showed the strongest association with schizophrenia (Table). Based on these results, we attempted to evaluate its role in schizophrenia. We examined the GWAS signal at the 3 genes encoding miR-9-5p (eFigure 3 in the Supplement). *MIR9-2*, the most highly expressed gene in BrainSpan of the 3 genes and the only one expressed in neuronal progenitor cells,[33] is contained within the $r^2 = 0.6$ clump of rs181900, an SNP just shy of significance in PGC2 ($P = 7.1 \times 10^{-8}$ including replication). For the 50 most schizophrenia-associated genes targeted by miR-9-5p, we provide an overview in eTable 11 in the Supplement. Among those are 21 genes residing in regions with a genome-wide-significant signal in the PGC2 GWAS.[12] Functional annotation of the miR-9-5p gene set using DAVID showed enrichment in regulatory function, brain development, and various transcription factors, with FOXO3 having the highest fold enrichment (eTable 12, eTable 13, and eTable 14 in the Supplement). In our analyses of spatiotemporal expression patterns, miR-9-5p showed a distinct peak in expression around the 16th postconception week across all brain regions (eFigure 4 in the Supplement).

To find a more homogeneous subset of miR-9-5p targets, we performed cluster analyses using brain expression data from BrainSpan.[27] We identified a cluster of 497 genes (cluster 4; eAppendix 2 and eTable 15 in the Supplement) that subsequently was shown to be enriched for protein-protein interactions ($P = 3 \times 10^{-5}$ for enrichment; eAppendix 2 and eFigure 5 in the Supplement). More importantly, however, it was enriched for schizophrenia risk genes compared with randomly drawn subsets of the original set of miR-9-5p targets ($P = 5 \times 10^{-3}$ for enrichment; eAppendix 2 in the Supplement). Cluster 4 was also enriched in rare variant analyses using summary statistics from a recently published schizophrenia exome-sequencing study[28] (1-sided binominal test, minor allele frequency <0.1%, disruptive mutations; $P = .013$; 194 vs 153 mutations). This was not the case for the full miR-9-5p targetome ($P = .10$; 524 vs 487 mutations). We also examined the miR-9-5p targetome expression in postmortem brains,[30] but the targets in the full set and cluster 4 showed only nominally significant module enrichments (eAppendix 2 in the Supplement).

Overlap in Targetomes of miR-9-5p and miR-137

Consistent with its previous implication in schizophrenia, we identified miR-137 among our top-ranking miRNAs (Table). During our cluster analysis for the top 10 miRNAs, we found that the targetomes of miR-137 and miR-9-5p clustered together and shared 231 predicted target genes (eFigure 2 in the Supplement). This overlap may a priori seem to be larger than what can be expected by chance. However, miRNAs have a markedly skewed distribution of number of genes they target, and correcting for this reveals the overlap to be nonsignificant ($P = .28$; eAppendix 2 and eFigure 6 in the Supplement).

Targeted Gene Set Enrichment Analyses

A total of 43 mature miRNAs were found to be located in schizophrenia GWAS loci (**Box**). However, the targetomes of these miRNAs did not show consistent association with schizophrenia (eTable 16 in the Supplement). Compared with our analysis for conserved miRNAs, the TargetScan gene set for miR-137 tested here, which included all targets regardless of conservation, showed a less significant association. A total of 17 mature miRNAs were found to be located in schizophrenia-associated CNVs (Box). For the targetomes of these miRNAs, miR-185-5p, located in the 22q11.21 deletion, showed the most consistent association with schizophrenia (eTable 17 in the Supplement).

The miR-137 locus on chromosome 1 also contains the high-confidence miRNA gene *MIR2682* just 719 base pairs downstream of *MIR137*. The targetome of miR-2682-5p was nominally significant at the lowest threshold using target predictions from TargetScan (eTable 16 in the Supplement). It has been shown that miRNAs closely located together often are coexpressed[34] and cotarget the same genes.[35] Analysis of *MIR137* and *MIR2682* expression using BrainSpan[27] shows that both miRNAs have similar spatiotemporal expression patterns with a peak in expression in early childhood (eFigure 4 in the Supplement). In addition, *MIR2682* is the gene that shows the highest degree of expressional correlation with *MIR137* among all genes in BrainSpan ($r = 0.679$). However, the overlap (n = 225) in targetomes of miR-2682-5p with miR-137 is not significantly larger than that of a random miRNA ($P = .31$; eAppendix 2 and eFigure 6 in the Supplement).

Discussion

We found evidence for an overall involvement of miRNAs in the etiology of multiple traits including schizophrenia. This finding is in line with results from a previous report that using GWAS data found enrichments of risk variants in miRNA genes and binding sites across multiple traits.[7] In contrast to this unspecific association, results of our gene set analyses in targetomes of conserved miRNAs revealed a more differentiated picture. Our results suggest the existence of several schizophrenia-associated miRNA targetomes, for which no evidence of association was found in additionally tested unrelated traits. In line with this, many of our top miRNAs are known to be brain specific and/or have known regulatory functions in the brain.[36-41]

The association of both miR-9-5p and its targetome marks our strongest finding. Intriguingly, a recent study identified miR-9-5p as the highest-abundance miRNA with significant differential expression (of 800 queried miRNAs). This result was found studying neuronal progenitor cells differentiated from human induced pluripotent stem cells from patients with schizophrenia (Kristen Brennand, PhD, and Gang Fang, PhD, written communication, October 2015).

Experimental evidence has implicated miR-9-5p as an important regulator of neuronal differentiation,[4,36] and it is predicted[13] and experimentally validated[42] that miR-9-5p targets the dopamine D_2 receptor, the predominant drug target

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> **Box. MicroRNAs From Genes in Genome-wide Association Study Loci and Copy Number Variations Associated With Schizophrenia**[a]
>
> **Schizophrenia Genome-wide Association Study Loci**
> miR-29b-2-5p, **miR-29b-3p**, **miR-29c-3p**, miR-29c-5p,
> **miR-33a-3p**, <u>miR-33a-5p</u>, **miR-33b-3p**, **miR-33b-5p**,
> <u>miR-130a-3p</u>, miR-130a-5p, **miR-137**, **miR-378i**, <u>miR-640</u>,
> miR-1228-3p, miR-1228-5p, <u>miR-1281</u>, **miR-1307-3p**, **miR-1307-5p**,
> **miR-2682-3p**, <u>**miR-2682-5p**</u>, <u>miR-3160-3p</u>, miR-3160-3p,
> miR-3160-5p, miR-3160-5p, miR-3655, miR-4301, miR-4304,
> miR-4529-3p, <u>miR-4529-5p</u>, miR-4655-3p, miR-4655-5p,
> <u>miR-4677-3p</u>, miR-4677-5p, miR-4688, miR-6773-3p,
> miR-6773-5p, miR-6777-3p, miR-6777-5p, miR-6843-3p,
> miR-6889-3p, miR-6889-5p, miR-8064, miR-8072
>
> **Schizophrenia Copy Number Variations**
> 1q21.1-Deletions: miR-6736-5p, miR-6736-3p
>
> 3q29-Deletions: <u>miR-922</u>
>
> 15q13.3-Deletions: miR-4509
>
> 16p11.2-Duplications: miR-3680-5p, miR-3680-3p
>
> 22q11.21-Deletions: miR-185-3p, <u>**miR-185-5p**</u>, miR-648,
> **miR-1306-3p**, **miR-1306-5p**, miR-3198, miR-3618, miR-4761-5p,
> miR-4761-3p, miR-6816-5p, miR-6816-3p
>
> [a] The following schizophrenia copy number variations did not contain any microRNA genes: NEDD4L-exonic duplication, 3q26.1 deletion, VIPR2 exonic duplication, C16orf72 exonic duplication, and 3q29 deletion. The microRNAs in bold are conserved and/or from a highly confident transcript. Names of microRNAs are underlined when the targetome for this microRNA showed a nominally significant association with schizophrenia using the top 1% of single-nucleotide polymorphisms and target predictions from TargetScan including nonconserved targets.

in schizophrenia.[43] Further of interest are the functional correlations with an additional target of miR-9-5p, the genome-wide–significant gene *FXR1*. Along with *FXR2*, *FXR1* is a homologue of the fragile X mental retardation 1 gene (*FMR1*), which itself is targeted by miR-9-5p. It has been shown that *FXR1* regulates the level of miR-9-5p and is necessary for efficient processing of pre-miR-9-5p.[44] Moreover, *FMR1* gene sets have shown association with schizophrenia in PGC2,[12] an exome-sequencing study,[28] and a CNV study.[45] Additionally, we used a framework of different approaches to expand our knowledge about the role of miR-9-5p and its targetome in the etiology of schizophrenia. The expression pattern for *MIR9-2* identified in our analyses is in agreement with the suggested neurodevelopmental role of this miRNA.[36] Despite strong evidence for importance of an identified subset of 497 genes in the miR-9-5p targetome, we were not successful in identifying a specific biological process that is connected to these genes. However, our identification of a coregulating function with *FOXO3* (a member of this cluster) is of interest as this gene fell just shy of genome-wide significance in PGC2 and plays a critical role in oxidative stress–induced neuronal cell death.[46]

Previously, *MIR137* has seen the highest degree of interest resulting from GWASs of schizophrenia. In this article, we demonstrated that another miRNA located in this schizophrenia hit region, *MIR2682*, is coexpressed with *MIR137* and shares part of its targetome. Further, the implication of miR-185-5p (22q11 microdeletion locus) and its targetome in schizophre-

nia is in line with results from a previous study that used an earlier, overlapping version of the PGC GWAS.[47]

Our study is not without limitations. Of particular concern are limitations pertaining to miRNA target prediction methods (eAppendix 2, eTable 1, eTable 5, and eTable 6 in the Supplement). Additionally, the sample size of the PGC2 study is still insufficient to detect all disease-associated variants at reasonable significance levels.[6] An additional limitation is our exclusion of the broad MHC region, which has potentially affected the identification of miRNAs mainly targeting genes in this region. Finally, our scoring-based approach, which was meant to rank the miRNA targetomes based on likelihood for their involvement in schizophrenia, could have prevented us from focusing on miRNAs with an important role in the etiology of schizophrenia. However, additional analyses with rank sum- or log sum–based scoring procedures revealed that miR-9-5p's leading position in our study was independent of the scoring function used (data not shown).

Moreover, our scoring approach was not intended to exclude other miRNAs from downstream analyses but to assist in the interpretation of our results (Table and eTable 1 in the Supplement). Future studies are warranted to illustrate the schizophrenia-related role of miRNAs in general and miR-9-5p's role in particular.

Conclusions

We used an analytical framework that broadly studied the role of miRNAs in common-variant schizophrenia susceptibility and found further evidence for their involvement. In particular, we identified a tripartite correlation between schizophrenia, miR-9-5p, and *FMR1/FXR1* with the corollary that establishing the functional overlaps and differences between *FMR1* and its homologues could potentially shed light on both the function of miR-9-5p and the etiology of schizophrenia.

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