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Review

MicroRNA dysregulation in schizophrenia

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ABSTRACT

Schizophrenia is a complex neuropsychiatric disorder that involves disturbances in neural circuitry and synaptic function. The exquisite network architecture and capacity for discreet post-synaptic remodeling of neurons requires coordination by an elaborate intracellular network of molecular signal transduction systems. The redundancy of these networks means that many combinations of gene variants have the potential to cause system dysfunction that manifest as related neurobehavioural syndromes. Recent investigation has revealed that posttranscriptional gene regulation and associated small non-coding microRNA (miRNA), are likely to be important factors shaping the topography of these networks. miRNA display complex temporospatial expression patterns in the mammalian brain and have the potential to regulate thousands of target genes by functioning as the specificity factor for intracellular gene-silencing machinery. They are emerging as key regulators of many neurodevelopmental and neurological processes as their dysregulation could lead to pervasive changes in the network structure during development and in the mature brain that are highly significant in the pathophysiology of schizophrenia. This review looks at mounting evidence that mature miRNA levels are altered in both the cerebral cortex and peripheral blood mononuclear cells (PBMCs) in schizophrenia. It also examines compelling evidence that the underlying miRNA biogenesis machinery and miRNA genes themselves are subject to disease-associated genetic mutation and epigenetic influence. Significantly, these changes in miRNA expression and associated machinery may represent new targets for pharmaceutical development, and the identification of miRNA signatures in PBMCs suggest that miRNA biomarkers of schizophrenia may also provide the basis for new clinical diagnostics. These developments have tremendous potential and highlight the significance of this avenue of research.

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Introduction

Schizophrenia is a debilitating psychotic disorder characterized by a diverse range of symptoms and cognitive impairments. While the neuropathology is relatively subtle and inconsistent at the anatomical, cellular and molecular levels, developments in neural imaging and histological techniques are refining our understanding of the spectrum of changes in gross anatomy, neural circuitry and cytoarchitecture. In recent years, the molecular neuropathology has also been revolutionized by the development of high-throughput genomics and proteomics. These allow a systems-level approach to understanding the role of molecular networks and interaction rather than individual candidate genes. A redundant pathways-based approach to the neurobiology of schizophrenia is also consistent with the genetics of the disorder, which is complex and polygenic. Many common gene variants, each with a weak effect size in combinatorial patterns of inheritance probably convey risk for the disorder through redundant pathways rather than as highly specific deficits. In this systems-view of schizophrenia, nodes of gene regulation will be particularly significant as they shape the topology of molecular pathways and networks and have the most influence on their behavior. These nodes can take many forms and interact at all levels of gene expression from DNA through to protein. While transcription factors and protein based signal transduction pathways are usually considered in this context, small non-coding RNA molecules known as microRNA (miRNA) have emerged as significant regulatory factors. These are expressed in a developmental and tissue-specific manner and are thought to regulate the majority of human genes (Lewis et al., 2005; Xie et al., 2005). Their influence, however, may be harder to trace than transcription factors and signal transduction molecules because their diverse interactions and capacity affect both RNA stability and translation. These small RNAs (18 to 26 nucleotides) are the specificity factors for a large multi-protein effector complex known as the RNA-induced silencing complex (RISC). The RISC, which is also responsible for the RNA interference pathway, interacts with its target genes through the sequence-specific base pairing, particularly at nucleotides 2 to 8 (seed region) and usually with the 3′UTR of specifically targeted mRNA (Gebauer et al., 2004; Lewis et al., 2005; Pillai et al., 2005) (Fig. 1). Although the first published account of a miRNA appeared nearly 20 years ago (Lee et al., 1993), it is only in more recent years that the diversity of these small regulatory RNA has been acknowledged. The widespread expression and activity of miRNA in the brain (Cao et al., 2006) support their implication in numerous neurological disorders.

Considering that each miRNA is potentially manipulating the expression of hundreds of target genes, the clinical implications of an abnormality or disturbance of this system are substantial, particularly if such abnormality occurs during development of the central nervous system. Studies have shown that the global depletion of Dicer in the brain is associated with dopaminergic behaviors and Parkinson's-like symptoms. Deletion of Dicer in dopamine neurons results in the progressive loss of these neurons and an increase in Parkinson's-like behaviors (such as reduced locomotion and increased immobility, behavioral changes and other neuronal defects) (Cuellar et al., 2008; Kim et al., 2007). miRNA expression has been shown to be altered in the midbrain of Parkinson's patients, suggesting that miRNA dysregulation could contribute to some aspects of this disease (Kim et al., 2007). In fragile X mental retardation, the gene responsible for the disorder FMR1, encodes the protein FMRP, which is present in synapses and is part of the RNA-induced silencing complex (RISC). Loss of FMRP impairs the function of RISC-mediated gene silencing, resulting in altered synaptic development (Jin et al., 2004). In Tourette's syndrome, a mutation in a miRNA binding site in the 3′UTR of SLITRK1 (a protein involved in neurite outgrowth) has been associated with the disorder (Abelson et al., 2005). Additionally, multiple studies have also suggested a link between miRNA dysregulation and Alzheimer's disease, although the specific molecular mechanisms remain unknown (Hebert et al., 2009; Nelson et al., 2010; Wang et al., 2010). In schizophrenia, miR-137 was recently shown to be highly associated ($p = 1.6 \times 10^{-11}$) with the disorder in one of the largest genome wide association studies conducted to date (Schizophrenia Psychiatric Genome-Wide Association Study GWAS Consortium, 2011).

miRNA can elicit a broad effect on gene expression and functional pathways and thus have important implications for neuropsychiatric disorders, such as schizophrenia, which have been characterized by a dysregulation of multiple pathways. These complex systems represent a significant challenge to both the investigation of the underlying biological causes and efforts to develop effective therapies. Examining the role of miRNA in coordinating signaling in psychiatric disease may explain both dysregulation of multiple pathways and offer a window to novel therapies that can target entire gene networks. Here we review the research data gathered from genetic, living donor, postmortem brain and animal studies, and discuss functional analysis and biological implications of miRNA dysregulation and pathophysiology. We also discuss the potential clinical utility of miRNA as novel biological markers and therapeutic targets for schizophrenia.

Evidence of miRNA dysregulation in schizophrenia

Postmortem analysis of cortical miRNA expression

Expression profiling studies in postmortem gray matter of individuals with schizophrenia have implicated numerous miRNA in the disorder. Utilizing a case–control approach, data has been compiled from various brain regions including the prefrontal cortex (BA9, BA10, BA46), parietal (BA7) and temporal cortices (BA22) using a variety of microarray platforms and quantitative RT-PCR (qPCR) techniques.

The first of these studies examined miRNA expression using a custom microarray (miRBase version 7.0) in total RNA extracted from postmortem dorsolateral prefrontal cortex (DLPFC) (BA9) (Perkins et al., 2007). This analysis of 13 schizophrenia samples and 21 controls identified 15 differentially expressed miRNA including 14 miRNA with decreased expression and one with increased expression in the schizophrenia group (Table 1). This was then validated by qPCR in a random selection of 4 cases and 4 controls. The qPCR was broadly consistent with the array with the exception of miR-7, indicating increased expression in the schizophrenia group. Using a very similar approach (Thomson et al., 2004), we conducted miRNA profiling analysis (miRBase v7.0) of the superior temporal gyrus (BA22) and reported a significant increase in expression of miR-181b and let-7b. We also confirmed differential expression of miR-181b by Northern blot and qPCR (Beveridge et al., 2008). Using an improved custom array (miRBase v7.1) printed with an LNA modified probe set including U6 small RNA controls, we reanalyzed an enlarged cohort ($n = 21$ pairs of cases and controls) and identified a further 59 mature miRNA upregulated in the schizophrenia group including miR-181b (Beveridge et al., 2008, 2010). As miRNA are extensively regulated through their biogenesis pathway in addition to underlying transcription (Thomson et al., 2006) we moved away from a global normalization strategy and used U6 snoRNA as reference. While there are risks of artifact in this approach, it provides a capacity to detect global changes in miRNA expression that are missed when miRNA expression is used as a normalization factor. These results were highly consistent with qPCR analysis of the entire cohort, and the schizophrenia-associated change in global miRNA expression was supported by analysis of uncapped RNA, which showed a significant increase in small RNAs (Beveridge et al., 2010). Similarly, we also reported significant upregulation of miRNA expression in DLPFC (BA9) with 26 miRNA displaying increased expression in the schizophrenia group (Beveridge et al., 2010). A large proportion of these samples (14 cases and 9 controls) were derived from the same individuals and miRNA expression was highly correlated between the two regions (unpublished). We also observed 11 miRNA displaying

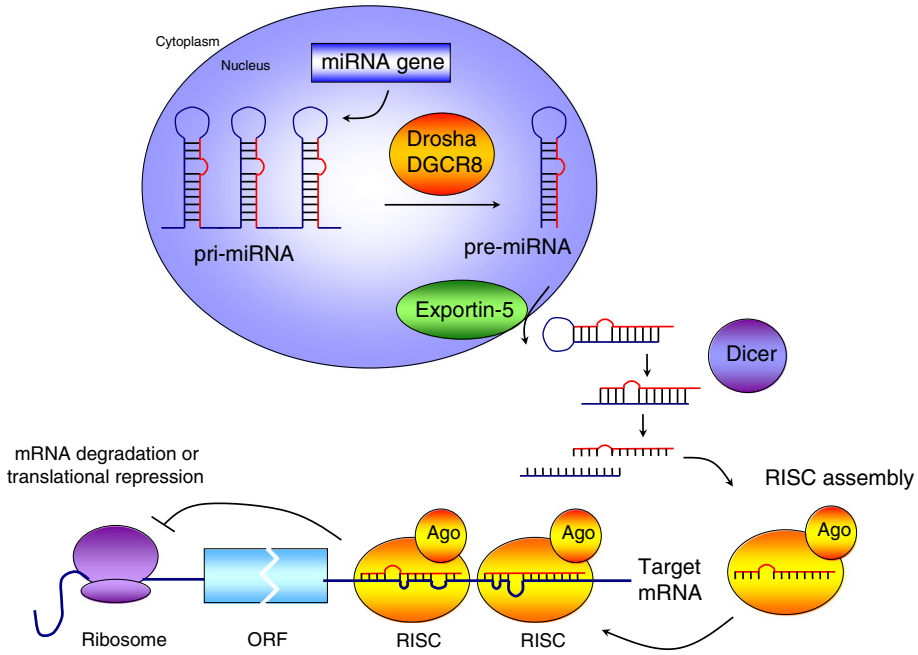

Fig. 1. Overview of miRNA processing. miRNA are encoded by their own genes or from the introns of protein-coding genes and are usually transcribed by RNA polymerase II. Inside the nucleus, the microprocessor complex (comprised of the ribonuclease Drosha and nuclear protein DGCR8) processes the primary miRNA transcripts into 70-nucleotide pre-miRNA. Exportin-5 then transports pre-miRNA into the cytoplasm where it is then processed into an RNA duplex by the ribonuclease Dicer. Although either strand of the duplex may potentially act as a functional miRNA, only one strand of the RNA duplex assembles into the RNA-induced silencing complex (RISC) with Argonaute 2 and consequently acts on its target gene to reduce mRNA and/or protein levels. ORF: open reading frame.

differential expression were in common between the two regions. The most striking feature conserved between both regions was the upregulation of miR-107 and the entire miR-15 family, which are predicted to share most of their target genes and would be expected to exert functionally convergent influence on cortical gene expression (Beveridge et al., 2010).

Mellios et al. (2009) utilized a frontal cortex (BA10) cohort to investigate the association between miRNA (miR-30a and miR-195) and schizophrenia candidate gene BDNF protein as well as neuropeptide Y, somatostatin, and parvalbumin mRNAs. It was reported that miR-195 expression, a member of the miR-15 family, shown to be upregulated in schizophrenia (Beveridge et al., 2010), was inversely associated with BDNF protein. This study also suggested that miR-195 might operate as a fine-tuner of the BDNF protein, which consequently could contribute to the prefrontal deficits in some GABAergic mRNAs. In another study by Mellios et al. (2010), the expression of the estrogen-sensitive miR-30b was determined in the prefrontal (BA10) and parietal (BA7) cortices. It was reported that female schizophrenia subjects displayed reduced levels of mature miR-30b transcripts while primary and precursor transcripts were unchanged.

The glutamate receptor ionotropic delta 1 (GRID1), gene previously implicated in schizophrenia (Fallin et al., 2005; Guo et al., 2007), also encodes the intronic precursor for miR-346. In view of this, Zhu et al. (2009) sought to investigate both the corresponding mature mRNA transcript and miRNA in a cohort of schizophrenia and bipolar subjects. The expression of miR-346 and GRID1 was shown to be reduced in schizophrenia subjects, though GRID1 was slightly above the threshold for significance ($p = 0.08$). Interestingly, miR-346 and GRID1 expressions were highly correlated in the control and bipolar samples ($r^2 > 0.47$), but much less correlated in the schizophrenia samples ($r^2 = 0.17$). It was suggested that since miR-346 and GRID1 mRNA are derived from the same primary transcript, the difference in correlation most likely arises from altered post-transcriptional processing or decreased stability of the transcripts in schizophrenia.

Utilizing a high-throughput qPCR approach (TaqMan low density array), Kim et al. (2010) profiled miRNA expression (miRBase v13.0) in a cohort of schizophrenia and bipolar subjects (Stanley collection, DLPFC BA46). This analysis reported that 7 miRNA displayed increased expression in the schizophrenia group (miR-7, miR-34a, miR-132, miR-132*, miR-154*, miR-212 and miR-544). A total of 15 miRNA were altered in the bipolar group, though presented an even split of 8 downregulated and 7 upregulated miRNA. Differentially expressed miRNA were also subjected to single tube qPCR validation (normalized with respect to U48 snoRNA) with 9 out of 21 consistent with the array. Discordance between array and single tube qPCR was possibly due to differences in normalization strategy (median polishing approach in the array and conventional housekeeping genes in free PCR) or the effect of cDNA pre-amplification in array analysis. The trend toward upregulation in the schizophrenia group is consistent with our study of the same brain region by Santarelli et al. (2011) as well as that of the DLPFC (BA9) and temporal cortex by Beveridge et al. (2008, 2009).

In a large postmortem cohort consisting of 37 schizophrenia and 37 control samples (NSW, TRC) from the DLPFC (BA46), we reported that 25 miRNA displayed increased expression and 3 showed reduced expression in schizophrenia after correction for multiple testing (SAM) (Santarelli et al., 2011). In this study we utilized a commercial bead array platform (Illumina) and normalized miRNA expression against the expression of small nucleolar RNA (miRBase version 9.1). qPCR was also used to validate a subset of 6 out of 10 differentially expressed miRNA.

Moreau et al. (2011) also used a high-throughput qPCR approach (TaqMan human miRNA assay, miRBase v9.2) to examine miRNA expression in postmortem schizophrenia and bipolar disorder in DLPFC (BA9) tissue from the Stanley array collection ($n = 35$/group). Normalization was performed with respect to the geometric mean of three stably expressed snoRNAs. Using Bayesian network averaging, a total of 19 miRNA appeared to display altered expression in the schizophrenia group, 10 downregulated (miR-22, miR-33, miR-106b, miR-138, miR-151, miR-210, miR-324-3p, miR-338, miR-339 and miR-425) and 9 upregulated (miR-27b, miR-148b, miR-193b, miR-148b, miR-186, miR-190, miR-301, miR-99a and miR-545). Notably, the miRNA with increased in expression in the schizophrenia

Table 1
Schizophrenia-associated miRNA.

Study	Type	Brain region	Species	Schizophrenia-associated miRNA
Hansen et al. (2007)	miR-SNP	N/A	Human	miR-198, miR-206
Perkins et al. (2007)	Postmortem brain	DLPFC (BA9)	Human	[Upregulated] miR-106b, miR-7 [Downregulated] miR-26b, miR-30b, miR-29b, miR-195, miR-92, miR-30a, miR-30d, miR-20b, miR-29c, miR-29a, miR-212, miR-24, miR-30e, miR-9*
Beveridge et al. (2008)	Postmortem brain	STG (BA22)	Human	miR-181b
Stark et al. (2008)	22q11.2 hemizygous deletion	PFC and Hippocampus	Mouse	[Downregulated] miR-106b, miR-134, miR-140*, miR-151, miR-18, miR-185, miR-194, miR-194, miR-212, miR-224, miR-323, miR-325, miR-362, miR-383, miR-409, miR-422b, miR-491, miR-494, miR-532, miR-540, miR-669a, miR-673, miR-674, miR-674*, miR-708, miR-93,
Xu et al. (2008)	CNV	N/A	Human	[CNV gain] 14q32.13-32.2, 1p34.2, 6p22.3, 16p13.2, 12q24.23, 3p24.1, 3p26.1, 4q34.1, 16p13.2 [CNV loss] 22q11.21, 3q22.2, 9q23, 19q13.12, 5q31.1, 10q21.1
Beveridge et al. (2010)	Postmortem brain	STG (BA22)	Human	[Upregulated] let-7e, miR-107, miR-125b, miR-128a, miR-128b, miR-129, miR-130a, miR-133b, miR-138, miR-146b, miR-148a, miR-150, miR-152, miR-155, miR-15a, miR-15b, miR-16, miR-17-3p, miR-17-5p, miR-181b, miR-195, miR-197, miR-199a*, miR-19a, miR-20a, miR-222, miR-23a, miR-24, miR-26b, miR-26b, miR-27b, miR-28, miR-296, miR-328, miR-330, miR-335, miR-338, miR-339, miR-340, miR-373*, miR-381, miR-409-5p, miR-432*, miR-452*, miR-455, miR-484, miR-485-5p, miR-486, miR-487a, miR-489, miR-494, miR-499, miR-502, miR-517a, miR-517c, miR-518b, miR-519d, miR-520a*, miR-520 g, miR-9*, miR-99a
Beveridge et al. (2010)	Postmortem brain	DLPFC (BA9)	Human	[Upregulated] let-7d, miR-101, miR-105, miR-107, miR-126*, miR-128a, miR-153, miR-15a, miR-15b, miR-16, miR-16, miR-181a, miR-181b, miR-181b, miR-181d, miR-184, miR-195, miR-199a, miR-20a, miR-219, miR-223, miR-26b, miR-27a, miR-29c, miR-302a*, miR-302b*, miR-31, miR-33, miR-338, miR-409-3p, miR-512-3p, miR-519b, miR-7
Feng et al. (2009)	miR-SNP	N/A	Human	let-7f-2, miR-188-3p, pre-miR-18b, miR-325-3p, pre-miR-502, pre-miR-505, miR-509-3p, miR-510-3p, miR-660
Kocerha et al. (2009)	Pharmacological	PFC	Mouse	miR-219
Mellios et al. (2009)	Postmortem brain	Frontal cortex (BA10)	Human	[Downregulated] miR-30e, miR-195
Sun et al. (2009)	miR-SNP	N/A	Human	miR-502, miR-510
Tabares-Seisdedos et al. (2009)	CNV	N/A	Human	miR-124-1, miR-320, miR-383, miR-486, miR-596, miR-597, miR-598,
Zhu et al. (2009)	Postmortem brain	DLPFC (BA46)	Human	miR-346
Kim et al. (2010)	Postmortem brain	DLPFC (BA46)	Human	[Upregulated] miR-132, miR-132*, miR-154*, miR-212, miR-34a, miR-544, miR-7
Mellios et al. (2010)	Postmortem brain	Frontal cortex (BA10)	Human	[Downregulated] miR-30b
Xu et al. (2010)	miR-SNP	N/A	Human	miR-24, pre-miR-30e, miR-30e
Gardiner et al. (2011)	Peripheral tissue (PBMC)	N/A	Human	[Downregulated] miR-107, miR-1275, miR-128, miR-130b*, miR-134, miR-148b, miR-150*, miR-151-3p, miR-16-2*, miR-181a, miR-200c, miR-224, miR-28-3p, miR-28-5p, miR-29b-1*, miR-30e*, miR-31, miR-329, miR-335*, miR-342-5p, miR-409-3p, miR-431, miR-432, miR-486-3p, miR-487b, miR-544, miR-574-3p, miR-576-5p, miR-584, miR-625*, miR-664, miR-877, miR-99b,
Lai et al. (2011)	Peripheral tissue (PBMC)	N/A	Human	[Upregulated] miR-34a, miR-449a, miR-548d, miR-564, miR-572, miR-652, [Downregulated] miR-432
Moreau et al. (2011)	Postmortem brain	DLPFC (BA9)	Human	[Upregulated] miR-148b, miR-151 miR-27b, miR-301, miR-545, miR-639 [downregulated] miR-106b, miR-138, miR-193b, miR-210, miR-22, miR-324-3p, miR-338, miR-339, miR-425
Santarelli et al. (2011)	Postmortem brain	DLPFC (BA46)	Human	[Upregulated] miR-105, miR-134, miR-148b, miR-150, miR-152, miR-154, miR-17-5p, miR-187, miR-193a, miR-199a*, miR-199b, miR-222, miR-25, miR-328, miR-382, miR-409-3p, miR-423, miR-425-5p, miR-433, miR-452*, miR-487a, miR-495, miR-502, miR-512-3p, miR-519c, miR-532, miR-542-3p, miR-548b, miR-590, miR-592, miR-652, miR-767-5p, miR-92b,
The Schizophrenia Psychiatric Genome-Wide Association Study GWAS Consortium (2011)	GWAS	N/A	Human	miR-137

These miRNA were found to have altered expression in multiple studies and displayed consistent changes with regards to the direction of change. Abbreviations: miR-SNP; single nucleotide polymorphism within a microRNA, DLPFC; dorsolateral prefrontal cortex, BA; Brodmann's Area, STG; superior temporal gyrus, CNV; copy-number variant, PBMC; peripheral blood mononuclear cells, GWAS; genome-wide association study.

group, displayed a greater fold-change than those downregulated (up to a 10% reduction compared to a 10–20% increase). The liquid bead array-based FlexmiR v2 assay was used for cross-platform validation. Using 7 samples, 8/10 miRNA were shown to have expression patterns that correlated well between platforms ($r = 0.76–0.98$). While the bipolar groups differentially expressed miRNA were consistently downregulated, with 24 having significantly reduced expression, there was not enough similarity between the two psychotic illnesses, in our opinion, to suggest that there is a common miRNA deficit associated with psychosis.

Collectively this research has identified numerous miRNA associated with schizophrenia. However, while some have displayed consistent changes across independent studies, there are many others that have not. A total of 16 miRNA are associated with increased expression in multiple studies (miR-105, miR-128a, miR-15a, miR-15b, miR-16, miR-17, miR-199a*, miR-20a, miR-222, miR-34a, miR-452*, miR-486, miR-487a, miR-502, miR-652, miR-7) and 11 miRNA with decreased expression (miR-106b, miR-151, miR-20b, miR-224, miR-30a, miR-30b, miR-30d, miR-30e, miR-383, miR-432, miR-505) (Table 1 and Supplementary Table 1). These miRNA have been reported to alter expression in the same direction in two or more studies, or are associated with a miR-SNP or CNV. There are also a number of miRNA, which are consistently reported to display significantly altered expression, except in opposing directions (Supplementary Table 2). Some miRNA show consistent changes in postmortem studies, while being altered in the opposite direction in peripheral tissues. Some of these discrepancies

can be attributed to sources of technical variation such as RNA isolation methods (total RNA vs. small RNA enriched, electrophoresis vs. solvent extraction), amplification, array platform, chemistry and normalization methods. While biological variation arising from regional differences in cytoarchitecture and cellular composition are difficult to control, the influence of defined postmortem variables such as age, gender, postmortem interval and brain pH can be adjusted statistically.

The neuropathology of miRNA is an evolving science employing a wide variety of approaches for tissue collection/extraction, determination of miRNA expression, normalization and statistical analysis. In future studies it would be valuable to combine the analysis from multiple centers using a consistent approach and utilize sequence information, particularly for miRNA genes to stratify on the basis of genotype, for example, miR-137. Where possible, it will also be informative to investigate different brain regions, including subcortical nuclei derived from the same individual. This will establish the difference between brain regions and genetic background and the extent of miRNA associated neuropathology. It will also be important to refine the extraction of tissue through laser capture microdissection to determine the influence of, and ultimately control for white matter composition, cytoarchitecture and variance in neural cell populations, as these parameters all vary within the brain, between individuals and disease status. In view of these limitations in understanding the neuropathology schizophrenia, it will also be of great interest to identify disease-associated changes in miRNA expression beyond the brain. Peripheral tissue samples, such as blood are more homogenous and can be collected in large numbers from living patients. Early investigation of peripheral miRNA in schizophrenia and the potential for clinical application is discussed below.

Schizophrenia-associated peripheral miRNA expression

In view of the changes in cortical miRNA expression, their underlying regulation and genetic association with schizophrenia, particularly miR-137, it is plausible to suggest that disease associated changes in miRNA expression may also be detectible in peripheral tissues such as lymphocytes. If they are, then these expression signatures may say something about the underlying mechanism that is consistent with, or not otherwise observed in brain tissue. For example, they may have some relevance to other aspects of schizophrenia risk, such as the immune response to maternal infection or cytokine exposure during pregnancy. miRNA signatures are indicative of a functionally relevant biological state and therefore may have utility as biomarkers of the disease or disease associated sub-phenotypes. Diagnosis of schizophrenia is currently based exclusively on signs and symptoms and requires qualified psychiatric assessment. Diagnosis based on a molecular signature could serve to refine the diagnosis and in some cases accelerate diagnosis and facilitate earlier interventions particularly in remote areas where access to psychiatric services are limited. miRNA based biomarkers have already been shown to be useful in the clinical stratification of cancer and have even greater prognostic significance than mRNA, possibly because they are discrete functional entities. Peripheral miRNA expression has also been investigated in other neurological disorders. In Alzheimer's disease, miRNA analysis has revealed patterns of altered expression in peripheral blood mononuclear cells (PBMCs) (Schipper et al., 2007) as well as in the cerebrospinal fluid and brain (Cogswell et al., 2008). Disease-associated miRNA profiles have also been identified in relation to multiple sclerosis and its remission status (Cox et al., 2010). PBMCs in particular are an attractive alternative tissue for profiling disease in living patients at statistically robust numbers. This readily accessible tissue can reflect global disease-associated changes in an underlying genetic disorder and may have special significance where immunological risk factors are believed to be involved.

We recently investigated the expression profile of PBMCs from 112 patients with schizophrenia and 76 non-psychiatric controls. This revealed 83 significantly downregulated miRNA in the schizophrenia group, including a large subgroup of miRNA (20%) transcribed from a single imprinted locus at the maternally expressed DLK1-DIO3 region on chromosome 14q32 (Gardiner et al., 2011). Similarly, Lai et al. (2011) identified a seven-miRNA signature (hsa-miR-34a, miR-449a, miR-564, miR-548d, miR-572 and miR-652 upregulated; miR-432 downregulated) in an initial cohort of 30 patients with schizophrenia and 30 controls. This was then validated in an extended cohort of 60 schizophrenia patients, and 30 controls. Significantly, miR-432 displayed schizophrenia-associated decreased expression in both independent cohorts. The expression of some of these 7 miRNA also correlated with schizophrenia patients negative symptoms, neurocognitive dysfunction and mismatch negativity performance. Interestingly, miR-449a significantly correlated with most features from the Wisconsin Card Sorting Test suggesting it might be involved in the executive functioning of the brain. Both of these studies have identified peripheral patterns of miRNA that could have utility as biomarkers for schizophrenia and associated sub-phenotypes. However, as most of the subjects reported the use of medication and some exposure to drugs and alcohol, larger studies with more comprehensive investigation of these confounds are needed to define their influence on miRNA expression in this tissue.

Mechanisms for miRNA dysregulation in schizophrenia

Mutation, genetic association and the alteration of miRNA transcription and processing

miRNA genes are for the most part expressed from polymerase II promoters and are regulated by transcription factors and chromatin structure in a manner synonymous to that of mRNA. These transcripts are usually in the form of non-coding mRNAs or derived from the introns of protein-coding mRNA. As such, the extent of transcription will vary with respect to the prevailing regulatory environment and epigenetic status as it does with any gene. miRNA transcription will also be dependent on the integrity of conserved sequence motifs in their promoters and enhancer elements. After transcription, their processing into precursor, and mature miRNA will also be dependent on the integrity of sequences in the primary hairpin that guide the miRNA biogenesis machinery. All of these components of miRNA genes are mutable and can give rise to heritable changes in the genome with functional significance to diseases such as schizophrenia.

The first studies to specifically investigate the association of miRNA polymorphisms and schizophrenia examined 28 brain expressed miRNA in a Scandinavian cohort (Danish, Swedish Norwegian cases $n = 420/163/257$, controls $n = 1006, 177, 293$). Interestingly, two miR-SNPs were identified to be associated with schizophrenia, residing in miR-206 (Danish sample $p = 0.0021$) and miR-198 (Norwegian sample $p = 0.038$), however the functional significance of these polymorphisms was not determined (Hansen et al., 2007). In a similar manner, Sun et al. (2009) also identified two schizophrenia-associated miR-SNPs (in miR-510 and miR-502) in a male Caucasian cohort ($n = 193$ cases and 191 controls). These polymorphisms were also functionally significant and were associated with reduced miRNA processing and thus produced less precursor and mature miR-502/510 transcripts in vitro. In the case of miR-502 at least, the reduced processing is thought to be due to the SNP impairing Drosha cleavage. Feng et al. (2009) also tested the hypothesis is that ultra-rare variants in X-linked miRNAs may predispose to schizophrenia, consistent with reduced fertility and consequent elimination of high-risk X-linked mutations within a few generations (Rees et al., 2011). They identified 8 ultra-rare variants in 3 males with schizophrenia within miRNA precursors (pre-miR-18b, pre-miR-505 and pre-miR-502) and 5 within the mature transcripts (let-7f-2, miR-188-3p, miR-325-3p, miR-509-3p, miR-510-3p and miR-660). In a Chinese case–control study, a new variant was identified within pre-mir-30e. This combined with SNPs in the mature miR-30e and miR-24/MAPK14 displayed a weak gene–gene interaction for schizophrenia risk (Xu et al., 2010). Recently, Begemann et al. (2010) showed that miR-498 binds with increased affinity when the T allele is present in the 3′UTR

SNP within the complexin 2 gene (CPLX2) and likely affecting the expression of the gene in a genotype-dependent manner.

Perhaps the most exciting miRNA gene to show genetic association with schizophrenia is *MIR137*. The loci containing this gene at 1p21.3 (rs1625579) emerged with the strongest association to schizophrenia in the most recent GWAS (Schizophrenia Psychiatric Genome-Wide Association Study GWAS Consortium, 2011). The gene for this long non-coding RNA transcript (AK094607) is over 100 kb from any protein-coding gene but encodes the primary transcript for miR-137. The functional significance of this polymorphism with respect to miR-137 expression is not immediately obvious as it occurs within the intron of the parent transcript and it may be a marker SNP for other more functionally significant polymorphisms more proximal to the miRNA hairpin. Further work will be needed to establish the function of this mutation or associated polymorphisms with respect to miR-137. While this is the first report to implicate miR-137 in schizophrenia, this miRNA is expressed in the brain and has been shown to regulate adult neurogenesis and neuronal maturation (Smrt et al., 2010; Szulwach et al., 2010), mechanisms through which variation could contribute to altered brain function in schizophrenia. Interestingly a number of protein coding genes associated with schizophrenia in this and other studies are also predicted target genes of miR-137 (including transcription factor 4 (TCF4), calcium channel, voltage-dependent, L type, alpha 1C subunit (CACNA1C), CUB and Sushi multiple domains 1 (CSMD1) and C10orf26) and thus plausibly this gene could convey risk mediated by a number of genes through a disturbance of in miRNA function.

Alterations in miRNA Biogenesis

While miRNA are regulated at the level of transcription and are susceptible to cis-acting mutations like other genes (Han et al., 2006; Zeng et al., 2005a, 2005b), they are also themselves extensively post-transcriptionally regulated and are sensitive to changes in the miRNA biogenesis pathway (Blow et al., 2006; Kawahara et al., 2007; Thomson et al., 2006; Yang et al., 2006). In respect to this influence, there is mounting evidence that dysregulation of cellular miRNA processing and maturation can contribute to the pathophysiology of disease including neurological and neuropsychiatric diseases such as schizophrenia. In this context it is interesting that copy number variation and polymorphisms affecting genes in the miRNA biogenesis pathway are possibly overrepresented in schizophrenia and related neurobehavioural syndromes. The best characterized of these associations is the 22q11.2 microdeletion syndromes known as DiGeorge or Velo-Cardio-Facial Syndrome (VCFS). These syndromes have a broad range of symptoms including neurobehavioral problems and cognitive deficits. Approximately 30% of children with the deletion will develop schizophrenia as they enter adolescence or early adulthood, one of the highest risk factors for developing the disorder, after identical twins or having two parents with schizophrenia (Bassett et al., 2003; Murphy et al., 1999). It has also been shown that 22q11.2 microdeletions are responsible for introducing new cases of schizophrenia into the population, accounting for approximately 2% of sporadic schizophrenia cases (Karayiorgou et al., 1995; Xu et al., 2008). The most common deletion spans 3 Mb, but a nested 1.5 Mb deletion contains a number of schizophrenia-associated genes. This region is conserved within the syntenic region of mouse chromosome 16 and contains nearly all orthologues of the human genes. Stark et al. (2008) generated a mouse model carrying the equivalent human 1.5 Mb microdeletion. This resulted in a hemizygous deletion of the Dgcr8 gene (a component of the microprocessor complex in the miRNA biogenesis pathway) along with the downregulation of 25 brain-related miRNA. They report that abnormal miRNA biogenesis emerges because of haploinsufficiency of the Dgcr8 gene and contributes to the behavioral and neuronal deficits associated with the 22q11.2 microdeletion. More recently, two other groups have investigated Dgcr8$^{+/-}$ knockout mouse models to examine the effect of Dgcr8 deficiency on miRNA expression neuronal morphology. Schofield et al. (2011) reported reduced levels of Dgcr8 and miRNA in the mouse prefrontal cortex which emerged throughout development. Consequently there were altered electrical properties of layer V pyramidal neurons, reduced excitatory synaptic transmission and decreased complexity of the basal neurites. In accordance with this study, Fenelon et al. (2011) also showed that the Dgcr8$^{+/-}$ knockouts had altered electrical properties and smaller dendritic spines in later V pyramidal neurons. In addition, they also observed less layers II and IV neurons overall.

Other components of the miRNA biogenesis family including Dicer have also been found to be subjected to spontaneous copy number variation. In a genome-wide scan for de novo CNVs in sporadic schizophrenia, Xu et al. (2008) identified a duplication which encompassed the DICER1 gene and Tabares-Seisdedos et al. (2009) commented that chromosome 8p (which contains 7 miRNA) is a CNV hot-spot for schizophrenia and other disorders such as autism. Increased Dicer gene dosage is consistent with schizophrenia-associated changes in Dicer gene expression observed in the DLPFC and elevated mature miRNA expression (Beveridge et al., 2010; Santarelli et al., 2011).

Postmortem brain studies have also suggested altered miRNA biogenesis as a mechanism for miRNA dysregulation in their cohorts. Perkins et al. (2007) showed that several miRNA with reduced expression also displayed a reduction in the ratio of mature to primary transcript, suggesting that miRNA processing may be impaired. Also, in our studies of BA22/STG and BA9, precursor transcripts (but not primary) of some upregulated miRNA were also shown to have increased expression. This also highlighted altered miRNA biogenesis as a mechanism behind the increase in miRNA expression. This was supported by an increase in the DGCR8 mRNA in both regions (BA22/STG, BA9) including an increase in DICER mRNA in the DLPFC. Dicer mRNA was also significantly increased in the BA46 cohort, however when utilizing a paired statistical approach there was also a statistically significant increase in DGCR8 and Drosha mRNAs as well.

Pharmacological impact

A number of studies have evaluated the impact of various neuroleptic drugs on the expression of miRNA. This has particular relevance when interpreting the results of postmortem studies as most schizophrenia subjects have an extensive antipsychotic treatment history and the effects on miRNA expression are largely unknown. Perkins et al. (2007) examined miRNA expression in rats treated with haloperidol versus untreated controls and reported that 3 miRNA (miR-128a, miR-128b and miR-199a) were upregulated in the frontal cortex as a consequence of this treatment. Notably, each of these miRNA have been shown to have increased expression in the DLPFC (BA9) (miR-128a, miR-199a) and STG (miR-128a, miR-128b) of schizophrenia subjects with a haloperidol treatment history (Beveridge et al., 2010).

In a related study, Zhou et al. (2009) examined the effects of common bipolar treatments, lithium and sodium valproate on miRNA expression in the rat hippocampus. They reported a downregulation of let-7b, let-7c, miR-128a, miR-24a, miR-30c, miR-34a and miR-221 and an upregulation of miR-144, all behaving similarly in both treatments. Further analysis substantiated a link between miR-34a and its potential target, metabotropic glutamate receptor 7 (GRM7). This has a particular relevance to schizophrenia with GRM7 as a potential candidate gene and miR-34a displaying increased expression in the DLPFC (BA46)(Kim et al., 2010) and PBMCs (Lai et al., 2011) of schizophrenia subjects.

In a study examining the effects of the selective NMDA receptor antagonist dizocilpine/MK-801 in mice, miR-219 was shown to be reduced in the prefrontal cortex upon drug treatment (Kocerha et al., 2009). Suggestive of a role for miR-219 in NMDA signaling, in vivo inhibition of miR-219 in the mouse brain caused the upregulation of its target, calcium/calmodulin-dependent protein kinase II gamma subunit

(CAMKIIγ). Consequently, the abnormal expression of CAMKIIγ impaired NMDA receptor signaling and relevant behavioral responses were observed. They also report that the dizocilpine/MK-801-induced effects on miR-219 could be diminished by pretreating the mice with haloperidol and clozapine. miR-219 has been shown to have increased expression in the DLPFC (BA9) of schizophrenia subjects (Beveridge et al., 2010) and is consistent with the NMDA receptor hypoactivity hypothesis in schizophrenia.

Functional implications

Target prediction methods and pathway analyses

Several algorithms have been developed to predict miRNA binding sites within the 3′UTR of target genes, typically taking into account sequence complementarity, conservation and the thermodynamics of the miRNA:mRNA duplex. Some methods require strict complementarity within the seed region while others also assess the conservation of the predicted binding site as an added level of stringency. The most widely used prediction algorithms include miRanda (miRBase (Griffiths-Jones et al., 2006) and microrna.org (Enright et al., 2003)), DIANA microT (Kiriakidou et al., 2004), PicTar (Krek et al., 2005) and TargetScan/TargetScanS (Lewis et al., 2003, 2005). There are also meta-databases of miRNA prediction algorithms that enable application of multiple predictions such as, TargetCombo (Sethupathy et al., 2006) and miRGen (Megraw et al., 2007). These interfaces provide access to unions and intersections of these 4 most widely used target prediction programs. As miRNA prediction methods typically predict large numbers of target genes, we incorporate a multi-hit approach that enriches for target genes that contain three or more target sites for the altered miRNA (Gardiner et al., 2011; Santarelli et al., 2011). Supporting this method, Hon et al. (2007) proposed that target genes with more putative miRNA binding sites are expected to have more potential for post-transcriptional regulation and greater intensity than those with only one or two sites. This approach to filtering target genes based on the number of putative interactions, provides important strategy for integrating the influence of changes in multiple miRNA that can collectively implicate many thousands of target genes. This is particularly important when it comes to making sense of the combined effect of multiple miRNA in pathway analysis.

To gain some appreciation of the biological implications of altered miRNA expression in schizophrenia, many groups have examined predicted miRNA targets and their associated pathways using resources such as the Database for Annotation, Visualization and Integrated Discovery (DAVID) (Dennis et al., 2003) and Ingenuity Pathway Analysis (IPA) (http://www.ingenuity.com/products/pathways_analysis.html). Postmortem studies of miRNA in schizophrenia have consistently reported the over-represented of the neurally significant KEGG pathways axon guidance, long term potentiation, focal adhesion and MAPK signaling (Beveridge et al., 2008, 2010; Gardiner et al., 2011; Perkins et al., 2007; Santarelli et al., 2011). Other groups that have utilized IPA for assessing affected pathways have also consistently highlighted nervous system development, function and disease as significant pathways represented by the miRNA target genes (Kim et al., 2010; Lai et al., 2011). These pathway analyses have highlighted nervous system development and processes involved in synaptic transmission as major effect points of altered miRNA expression. These observations are consistent with the disconnection hypothesis, which suggests that schizophrenia is a phenomenon of abnormal synaptic plasticity because of inefficient or inappropriate wiring of neural networks (Friston, 1998).

Biological approaches to target validation

One of the advantages in understanding miRNA function is that they interact with their mRNA targets through a somewhat predictable Watson–Crick, sequence-dependent manner. Despite the power of these bioinformatic approaches, they are prone to both false positives and false negatives. To gain further biological insight into the target genes of altered miRNA, in vitro assays have been established to measure endogenous target gene activity in response to miRNA transfection. This approach however does not distinguish between the post-transcriptional influences of the transfected miRNA from other secondary influences such as a change in transcription factors. To achieve a more direct assessment of the relationship between a miRNA and specific binding sites it is also important to investigate the gene-miRNA interaction by reporter gene assay. In these assays the target genes 3′UTR or specific miRNA recognition elements (MRE) from are cloned into the 3′UTR of a fluorescent or luminescent reporter gene such as green fluorescent protein or luciferase. As a control measure, mutations may also be introduced into the seed-pairing region of the binding site. The reporter gene activity, typically luciferase, is then measured in response to transfection of synthetic miRNA agonist (miRNA mimic) or antimiR antisense-antagonist (antagomiR). Evidence of a canonical miRNA-mRNA interaction is supported by the observation of an inverse relationship between the miRNA concentration and reporter activity. This is demonstrated by a decrease in reporter gene expression in response to miRNA transfection or an increase in expression in response to antimiR transfection that can suppress endogenous miRNA activity.

This approach was utilized by Mellios (2008) to substantiate the miRNA-target gene relationship between BDNF and schizophrenia-associated miRNA, miR-30a and miR-195 (Table 2). Relative luciferase activity was reduced upon transfection with synthetic versions of the precursor transcripts. This method allows the precursor transcripts to undergo processing by the endogenous miRNA pathway before eliciting their actions. Likewise, Begemann et al. (2010) used this method to show that a cognition-relevant SNP (rs3822674) influenced the binding of miR-498 within the CPLX2 3′UTR and expression of the gene. We also used this approach to investigate a number of genes associated with synaptic function that were predicted targets of functionally conserved miR-107 and the miR-15 family, dysregulated in both the STG (BA22) and DLPFC (BA9) (Beveridge et al., 2010) as well as DLPFC (BA46) in the case of miR-107 (Beveridge et al., 2010; Santarelli et al., 2011). Since these miRNA share a highly similar seed region, there was substantial overlap in predicted target genes (including numerous schizophrenia candidate genes). A number of these targets were tested against the miR-15 family members and miR-107 in response to transfection with synthetic versions of the mature miRNA as well as antimiRs. The most consistently responsive target MREs were derived from the 3′UTR of the schizophrenia candidate genes GRIN3A and RELN, and miR-107 appeared to have the greatest overall effect. A number of miRNA-target gene pairs displayed significant bi-directional modulation, responding as expected to both miRNA and anti-miR transfection (Table 2). Using the same approach we also reported that schizophrenia-associated miR-181b was able to repress VSNL1 and GRIA2 expression (Beveridge et al., 2008).

Summary and future prospects

Schizophrenia has a complex neurobehavioural phenotype that is thought to develop through disturbances in neural circuitry and synaptic function. The complex mechanism and architecture of synapses in the human brain requires coordination of an equally elaborate intracellular network of molecular signal transduction systems. The redundancy of these networks means that many combinations of gene variants can give rise to system dysfunction that manifest as related neurobehavioural syndromes. Posttranscriptional gene regulation and small non-coding RNA are likely to be important factors shaping the topography of this matrix. They are expressed in large numbers in the brain and are emerging as key regulators of many neurodevelopmental and neurological processes. Their dysregulation during development

Table 2
Supported schizophrenia-relevant miRNA/target relationships by reporter gene assay.

	Schizophrenia-relevant target gene	Reference
miRNA suppression of gene targets		
miR-30a	BDNF	Mellios (2008)
miR-195	BDNF	Mellios (2008)
miR-195	RGS4, GRIN3A, RELN	Beveridge et al. (2010)
miR-107	DRD1	Beveridge et al. (2010)
miR-15a	RGS4, GRM7, GRIN3A, VSNL1	Beveridge et al. (2010)
miR-15b	RGS4, GRM7, HTR2A, RELN	Beveridge et al. (2010)
miR-16	RGS4, GRIN3A, RELN, VSNL1, DLG4	Beveridge et al. (2010)
miR-498	CPLX2	Begemann et al. (2010)
Bi-directional modulation (both miRNA and anti-miR)		
miR-181b	GRIA2, VSNL1	Beveridge et al. (2008)
miR-107	RGS4, GRIN3A, RELN	Beveridge et al. (2010)
miR-15a	GRIN3A, RELN	Beveridge et al. (2010)
miR-15b	GRM7	Beveridge et al. (2010)
miR-219	CaMKIIγ	Kocerha et al. (2009)

Gene abbreviations: BDNF: brain-derived neurotrophic factor, RGS4: regulator of G-protein signaling 4, GRIN3A: glutamate [NMDA] receptor subunit 3A, RELN: reelin, DRD1: dopamine receptor D1, GRM7: glutamate receptor metabotropic 7, VSNL1: visinin-like 1, DLG4: disks large homolog 4 (also known as PSD-95 post synaptic density 95), GRIA2: glutamate receptor ionotropic AMPA 2.

and in the mature brain could lead to pervasive changes in the network structure that are difficult to understand but highly significant to the underlying pathophysiology and neuropathology of schizophrenia. This review has cataloged mounting evidence that mature miRNA levels are altered in both the cerebral cortex and peripheral blood mononuclear cells. We have also examined evidence that suggests underlying miRNA biogenesis machinery and the miRNA genes themselves are subject to genetic mutation and epigenetic influence. These are exemplified by CNVs and SNPs in the DGCR8/22q11 region and miR-137 respectively, which are both strongly associated with schizophrenia. Future work should hopefully lead to more consensuses in the neuropathology, perhaps by consideration of both the specific genetic and broader posttranscriptional underlying mechanisms regulating miRNA expression. With so little known about the function of individual miRNA and the biological implications of their dysregulation in the developing and mature brain, it will be important to characterize these molecules better at the molecular, cellular, developmental and ultimately neurobehavioural levels. At one end of this spectrum, there are a number of high throughput methodologies that are unlocking the mysteries of miRNA targeting, while at the other end, several model systems including transgenic zebrafish and rodent models that are taking up the challenge of investigating the role of miRNA in development and behavior. These model systems not only provide the basis for understanding the phenotype of miRNA dysfunction, they provide a platform for the investigating therapeutics. miRNA and the underlying miRNA biogenesis machinery are potentially both novel drug targets and new drug entities in the fight to control neuropsychiatric conditions. With the identification of miRNA biomarkers in PBMCs and other peripheral sources, the clinical development of miRNA oriented pharmaceutical interventions may be accompanied by miRNA-based clinical diagnostics. New biomarkers for schizophrenia and associated phenotypes could ultimately provide the basis for earlier detection, disease stratification and the prediction of response to drugs and side effects. Gathering a better appreciation of the role of miRNA in the brain and in neuropsychiatric disorders such as schizophrenia is an enormous challenge, but the potential significance of these discoveries are likely to be highly significant.

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Appendix A. Supplementary data

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References

Abelson, J.F., et al., 2005. Sequence variants in SLITRK1 are associated with Tourette's syndrome. Science 310, 317–320.
Bassett, A.S., et al., 2003. The schizophrenia phenotype in 22q11 deletion syndrome. Am. J. Psychiatry 160, 1580–1586.
Begemann, M., et al., 2010. Modification of cognitive performance in schizophrenia by complexin 2 gene polymorphisms. Arch. Gen. Psychiatry 67, 879–888.
Beveridge, N.J., et al., 2008. Dysregulation of miRNA 181b in the temporal cortex in schizophrenia. Hum. Mol. Genet. 17, 1156–1168.
Beveridge, N.J., et al., 2010. Schizophrenia is associated with an increase in cortical microRNA biogenesis. Mol. Psychiatry 15, 1176–1189.
Blow, M.J., et al., 2006. RNA editing of human microRNAs. Genome Biol. 7, R27.
Cao, X., et al., 2006. Noncoding RNAs in the mammalian central nervous system. Annu. Rev. Neurosci. 29, 77–103.
Cogswell, J.P., et al., 2008. Identification of miRNA changes in Alzheimer's disease brain and CSF yields putative biomarkers and insights into disease pathways. J. Alzheimers Dis. 14, 27–41.
Cox, M.B., et al., 2010. MicroRNAs miR-17 and miR-20a inhibit T cell activation genes and are under-expressed in MS whole blood. PLoS One 5, e12132.
Cuellar, T.L., et al., 2008. Dicer loss in striatal neurons produces behavioral and neuroanatomical phenotypes in the absence of neurodegeneration. Proc. Natl. Acad. Sci. U. S. A. 105, 5614–5619.
Dennis Jr., G., et al., 2003. DAVID: database for annotation, visualization, and integrated discovery. Genome Biol. 4, P3.
Enright, A.J., et al., 2003. MicroRNA targets in Drosophila. Genome Biol. 5, R1.
Fallin, M.D., et al., 2005. Bipolar I disorder and schizophrenia: a 440-single-nucleotide polymorphism screen of 64 candidate genes among Ashkenazi Jewish case-parent trios. Am. J. Hum. Genet. 77, 918–936.
Fenelon, K., et al., 2011. Deficiency of Dgcr8, a gene disrupted by the 22q11. 2 microdeletion, results in altered short-term plasticity in the prefrontal cortex. Proc. Natl. Acad. Sci. U. S. A. 108, 4447–4452.
Feng, J., et al., 2009. Evidence for X-Chromosomal Schizophrenia Associated with microRNA Alterations. PLoS One 4, e6121.
Friston, K.J., 1998. The disconnection hypothesis. Schizophr. Res. 30, 115–125.
Gardiner, E., Beveridge, N.J., Wu, J.Q., Carr, V., Scott, R.J., Tooney, P.A., Cairns, M.J., 2011. Imprinted DLK1-DIO3 region of 14q32 defines a schizophrenia-associated miRNA signature in peripheral blood mononuclear cells. Mol. Psychiatry 2011 Jul 5. doi:10.1038/mp.2011.78.
Gebauer, F., et al., 2004. Molecular mechanisms of translational control. Nat. Rev. Mol. Cell Biol. 5, 827–835.
Griffiths-Jones, S., et al., 2006. miRBase: microRNA sequences, targets and gene nomenclature. Nucleic Acids Res. 34, D140–D144.
Guo, S.Z., et al., 2007. A case–control association study between the GRID1 gene and schizophrenia in the Chinese Northern Han population. Schizophr. Res. 93, 385–390.
Han, J., et al., 2006. Molecular basis for the recognition of primary microRNAs by the Drosha-DGCR8 complex. Cell 125, 887–901.
Hansen, T., et al., 2007. Brain expressed microRNAs implicated in schizophrenia etiology. PLoS One 2, e873.
Hebert, S.S., et al., 2009. Alterations of the microRNA network cause neurodegenerative disease. Trends Neurosci. 32, 199–206.
Hon, L.S., et al., 2007. The roles of binding site arrangement and combinatorial targeting in microRNA repression of gene expression. Genome Biol. 8, R166.
Jin, P., et al., 2004. RNA and microRNAs in fragile X mental retardation. Nat. Cell Biol. 6, 1048–1053.
Karayiorgou, M., et al., 1995. Schizophrenia susceptibility associated with interstitial deletions of chromosome 22q11. Proc. Natl. Acad. Sci. U. S. A. 92, 7612–7616.
Kawahara, Y., et al., 2007. RNA editing of the microRNA-151 precursor blocks cleavage by the Dicer-TRBP complex. EMBO Rep. 8, 763–769.
Kim, J., et al., 2007. A MicroRNA feedback circuit in midbrain dopamine neurons. Science 317, 1220–1224.
Kim, A.H., et al., 2010. MicroRNA expression profiling in the prefrontal cortex of individuals affected with schizophrenia and bipolar disorders. Schizophr. Res. 124, 183–191.
Kiriakidou, M., et al., 2004. A combined computational-experimental approach predicts human microRNA targets. Genes Dev. 18, 1165–1178.
Kocerha, J., et al., 2009. MicroRNA-219 modulates NMDA receptor-mediated neurobehavioral dysfunction. Proc. Natl. Acad. Sci. 106, 3507–3512.
Krek, A., et al., 2005. Combinatorial microRNA target predictions. Nat. Genet. 37, 495–500.
Lai, C.-Y., et al., 2011. MicroRNA expression aberration as potential peripheral blood biomarkers for schizophrenia. PLoS One 6, e21635.
Lee, R.C., et al., 1993. The C. elegans heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. Cell 75, 843–854.

Lewis, B.P., et al., 2003. Prediction of mammalian microRNA targets. Cell 115, 787–798.
Lewis, B.P., et al., 2005. Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets. Cell 120, 15–20.
Megraw, M., et al., 2007. miRGen: a database for the study of animal microRNA genomic organization and function. Nucleic Acids Res. 35, D149–D155.
Mellios, N., 2008. A set of differentially expressed miRNAs, including miR-30a-5p, act as post-transcriptional inhibitors of BDNF in prefrontal cortex - Supp. Material. Hum. Mol. Genet. 1–17.
Mellios, N., et al., 2009. Molecular determinants of dysregulated GABAergic gene expression in the prefrontal cortex of subjects with schizophrenia. Biol. Psychiatry 65, 1006–1014.
Mellios, N., et al., 2010. Gender-Specific reduction of estrogen-sensitive small RNA, miR-30b, in subjects with schizophrenia. Schizophr. Bull.
Moreau, M.P., et al., 2011. Altered microRNA expression profiles in postmortem brain samples from individuals with schizophrenia and bipolar disorder. Biol. Psychiatry 69, 188–193.
Murphy, K.C., et al., 1999. High rates of schizophrenia in adults with velo-cardio-facial syndrome. Arch. Gen. Psychiatry 56, 940–945.
Nelson, P.T., et al., 2010. MiR-107 is reduced in Alzheimer's disease brain neocortex: validation study. J. Alzheimers Dis. 21, 75–79.
Perkins, D.O., et al., 2007. microRNA expression in the prefrontal cortex of individuals with schizophrenia and schizoaffective disorder. Genome Biol. 8, R27.
Pillai, R.S., Bhattacharyya, S.N., Artus, C.G., Zoller, T., Cougot, N., Basyuk, E., et al., 2005. Inhibition of translational initiation by Let-7 MicroRNA in human cells. Science 309 (5740), 1573–1576.
Rees, E., Moskvina, V., Owen, M.J., O'Donovan, M.C., Kirov, G., 2011. De novo rates and selection of schizophrenia-associated copy number variants. Biol Psychiatry 70 (12), 1109–1114.
Santarelli, D.M., et al., 2011. Upregulation of dicer and MicroRNA expression in the dorsolateral prefrontal cortex Brodmann Area 46 in schizophrenia. Biol. Psychiatry 69, 180–187.
Schipper, H.M., et al., 2007. MicroRNA expression in Alzheimer blood mononuclear cells. Gene Regul. Syst. Bio. 1, 263–274.
Schizophrenia Psychiatric Genome-Wide Association Study GWAS Consortium, 2011. Genome-wide association study identifies five new schizophrenia loci. Nat. Genet. 1–10.
Schofield, C.M., et al., 2011. Monoallelic deletion of the microRNA biogenesis gene Dgcr8 produces deficits in the development of excitatory synaptic transmission in the prefrontal cortex. Neural Dev. 6, 11.
Sethupathy, P., et al., 2006. A guide through present computational approaches for the identification of mammalian microRNA targets. Nat. Methods 3, 881–886.
Smrt, R.D., et al., 2010. MicroRNA miR-137 regulates neuronal maturation by targeting ubiquitin ligase mind bomb-1. Stem Cells 28, 1060–1070.
Stark, K.L., et al., 2008. Altered brain microRNA biogenesis contributes to phenotypic deficits in a 22q11-deletion mouse model. Nat. Genet. 40, 751–760.
Sun, G., et al., 2009. SNPs in human miRNA genes affect biogenesis and function. RNA 15, 1640–1651.
Szulwach, K.E., et al., 2010. Cross talk between microRNA and epigenetic regulation in adult neurogenesis. J. Cell Biol. 189, 127–141.
Tabares-Seisdedos, R., et al., 2009. Chromosome 8p as a potential hub for developmental neuropsychiatric disorders: implications for schizophrenia, autism and cancer. Mol. Psychiatry 14, 563–589.
Thomson, J.M., et al., 2004. A custom microarray platform for analysis of microRNA gene expression. Nat. Methods 1, 47–53.
Thomson, J.M., et al., 2006. Extensive post-transcriptional regulation of microRNAs and its implications for cancer. Genes Dev. 20, 2202–2207.
Wang, W.X., et al., 2010. miR-107 regulates granulin/progranulin with implications for traumatic brain injury and neurodegenerative disease. Am. J. Pathol. 177, 334–345.
Xie, X., et al., 2005. Systematic discovery of regulatory motifs in human promoters and 3′ UTRs by comparison of several mammals. Nature 434, 338–345.
Xu, B., et al., 2008. Strong association of de novo copy number mutations with sporadic schizophrenia. Nat. Genet. 40, 880–885.
Xu, Y., et al., 2010. MicroRNAs and target site screening reveals a pre-microRNA-30e variant associated with schizophrenia. Schizophr. Res. 119, 219–227.
Yang, W., et al., 2006. Modulation of microRNA processing and expression through RNA editing by ADAR deaminases. Nat. Struct. Mol. Biol. 13, 13–21.
Zeng, Y., et al., 2005a. Efficient processing of primary microRNA hairpins by Drosha requires flanking nonstructured RNA sequences. J. Biol. Chem. 280, 27595–27603.
Zeng, Y., et al., 2005b. Recognition and cleavage of primary microRNA precursors by the nuclear processing enzyme Drosha. EMBO J. 24, 138–148.
Zhou, R., et al., 2009. Evidence for selective microRNAs and their effectors as common long-term targets for the actions of mood stabilizers. Neuropsychopharmacology 34, 1395–1405.
Zhu, Y., et al., 2009. A MicroRNA gene is hosted in an intron of a schizophrenia-susceptibility gene. Schizophr. Res. 1–4.