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microRNAs in Psychiatric and Neurodevelopmental Disorders

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Abstract

Abnormalities in microRNA (miRNA)-mediated gene regulation have been observed in a variety of human diseases, especially in cancer. Here, we provide an account of newly emerging connections between miRNAs with various psychiatric and neurodevelopmental disorders, including recent findings of miRNA dysregulation in the 22q11.2 microdeletion syndrome, a well-established genetic risk factor for schizophrenia. miRNAs appear to be components of both the genetic architecture of these complex phenotypes as well as integral parts of the biological pathways that mediate the effects of primary genetic deficits. Therefore, they may contribute to both genetic heterogeneity and phenotypic variation of psychiatric and neurodevelopmental disorders and could serve as novel therapeutic targets.

Keywords

microRNA; psychiatric disorder; schizophrenia; chromosome 22q11.2; mental retardation; autism; neurodevelopment; synaptic plasticity

1. Introduction

MicroRNAs (miRNAs) are a class of small non-coding RNAs about 22 nucleotides long, generated through a series of cleavage steps from long precursor RNA transcripts. Binding of miRNAs to mRNAs results in degradation of mRNA targets or repression of their translation. Since the discovery of the first miRNA, lin-4, as a critical modulator of developmental timing in the nematode *C. elegans* (Lee et al., 1993), this class of non-coding genes has been associated with many critical physiological processes and is thought to be an important component of the post-transcriptional regulatory machinery. Consistent with this notion, abnormalities in miRNA expression and miRNA-mediated gene regulation have been observed in a variety of human diseases, such as cancer, heart disease and viral infection.

The role that miRNAs play in the central nervous system is the subject of active investigation. miRNAs present a number of attractive properties that promise to provide important insights

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into the complexity underlying neuronal development and function. First, over half of the miRNAs identified to-date are highly or exclusively expressed in the brain, and many of them have been implicated in many important aspects of neuronal function (Cao et al., 2006). Second, the fact that control of miRNA expression can be exerted at various levels (transcriptional and post-transcriptional) and at several different cellular locations offers a unique regulatory flexibility (Han et al., 2009; Hutvagner et al., 2001; Kadener et al., 2009; Lugli et al., 2008; Obernosterer et al., 2006; Ramachandran et al., 2008; Thomson et al., 2006; Triboulet et al., 2009; Wu et al., 2006). Third, miRNAs have regulatory properties that are distinct from other regulatory elements in the cells, such as transcription factors (Hobert, 2008). Indeed, unlike transcription factors, which can only function in the nucleus, miRNAs can bind to mRNAs in many different intracellular compartments and regulate their expression locally. Local protein synthesis at synaptic sites, for example, is very important for neuronal synaptic plasticity and several miRNAs have been shown to regulate this process (Schratt, 2009). Fourth, regulation of gene expression by miRNAs is a dynamic, reversible and combinatorial process. Notably, one miRNA might have multiple mRNA targets and different miRNAs can bind on the same mRNA target and modulate its levels in a synergistic manner. These properties might help miRNAs integrate different intracellular signals and/or coordinate different signaling pathways.

For all the reasons outlined above, considerable attention has been paid to the roles of miRNAs in the etiology and pathophysiology of psychiatric as well as neurodevelopmental disorders, including mental retardation (or intellectual disability) as well as autism and autism spectrum disorders. Here, we summarize and discuss current progress in this direction and highlight the emerging connections between miRNAs with neuropsychiatric and neurodevelopmental disorders (Fig. 1).

2. The impact of miRNA dysregulation in neural structure and function

The impact of miRNAs on both developing and mature nervous system has been assessed using a variety of strategies. miRNAs have been shown to play important roles in neural morphology and function as well as behavior. Below we discuss a few relevant examples that offer a context to understand the role of miRNAs in neuropsychiatric disorders and cognitive dysfunction. A more detailed account of the role of miRNAs in the nervous system can be found in recent excellent reviews of the relevant literature in this issue and elsewhere (Fineberg et al., 2009; Schratt, 2009).

2.1 Disrupting miRNA biogenesis and action

Most of the miRNAs share the same transcription machinery as mRNAs. They are transcribed by RNA polymerase II as long primary transcripts (pri-miRNAs) with 5'Cap and 3'poly(A) tails. Pri-miRNAs are then processed in the nucleus into stem-loop precursor miRNAs about 70 nucleotides long (pre-miRNAs) by the microprocessor (a complex containing type-III RNase Drosha and its partner protein Dgcr8). Pre-miRNAs are then exported to the cytoplasm, a process facilitated by Exportin-5. In the cytoplasm, pre-miRNAs are further cleaved into mature miRNA duplexes by another type-III RNase, Dicer. Dicer and several other RNA binding proteins, such as Ago2, PACT and TRBP, incorporate one strand of mature miRNA duplexes into the ribosome induced silencing complex (RISC). The miRNA-associated RISC binds to the target mRNA to inhibit its translation or cause the degradation of the target mRNA (Kim, 2005).

Disruptions of miRNA biogenesis and the RISC complex have provided important insight on the critical role of miRNAs on neuronal survival, development, differentiation and function in the central nervous system. For example, the first evidence that miRNAs are necessary for the normal development of the nervous system was provided by the knockout of the *Dicer* gene

in Zebrafish (Giraldez et al., 2005). *Dicer* mutants showed severe defects in neural tube morphogenesis arising from abnormal neuronal differentiation. In mammals, *Dicer*-deficient mice die at embryonic day 7.5 before neurulation occurs (Murchison et al., 2005). Conditional knockout of *Dicer* in the cortex and hippocampus disrupts morphogenesis of these regions (Davis et al., 2008). Progressive cell death was observed when *Dicer* was inactivated postnatally in the cerebellum (Schaefer et al., 2007) or in dopaminergic neurons in the forebrain (Kim et al., 2007). Because *Dicer* is involved in both miRNA biogenesis and siRNA machinery, it has been difficult to distinguish the effects of these two pathways. Nevertheless, consistent with the *Dicer* knockout models, disruption of *Dgcr8* gene (which should not affect the siRNA machinery) also resulted in deficits in neural development. *Dgcr8* homozygous knockout mice die at embryonic day 6.5 (Stark et al., 2008; Wang et al., 2007). *Dgcr8* heterozygous mice (*Dgcr8*^{+/-}) display partially impaired miRNA biogenesis accompanied by a number of neuronal and behavioral deficits (Stark et al., 2008) (see below). Finally, loss-of-function mutation of the *Drosophila* homologue of *AGO1*, *dAgo1* (one of the Argonaute proteins that facilitate the loading of miRNAs into the RISC) results in global developmental defects, with the most prominent malformation found in the nervous system (Kataoka et al., 2001). It has also been reported that *Ago2*-null mice die early in development and show severe neural tube defects (Liu et al., 2004).

2.2 Disrupting individual miRNAs

Insights into the critical role of miRNAs on neuronal development and function have also been provided by the analysis of individual miRNAs. For example, Schratt et al. showed that overexpression of miR-134 significantly decreased spine volume while overexpression of a 2'-*O*-methylated anti-miR-134 oligonucleotide increased spine width (Schratt et al., 2006). It was also shown that BDNF treatment can relieve miR-134-dependent translation inhibition of its target, *Limk1*, a kinase that regulates actin and microtubule polymerization, leading to increased synthesis of *Limk1* protein and to dendritic spine morphology changes. Further investigation indicated that the transcription of a large miRNA cluster including miR-134 can be induced by increasing neuronal activity in primary rat neurons. Myocyte enhancing factor 2 (*Mef2*) is necessary and sufficient for this control and it can induce expression of miR-134 and promote neurite outgrowth by inhibiting translation of the mRNA encoding for the translational repressor *Pumilio2* (Fiore et al., 2009). These studies suggest that miR-134 plays an important role in controlling neuronal morphology in response to external stimuli, such as neurotrophic factors and neuronal activity.

Another example is provided by miR-124a, one of the most abundant miRNAs in mammalian brain, accounting for 25%–48% of all mouse brain miRNAs (Lagos-Quintana et al., 2002). miR-124a is mainly expressed in differentiating and mature neurons (Deo et al., 2006) and participates in many aspects of neuronal differentiation and maturation by interacting with multiple targets. Ectopic expression of miR-124a in HeLa cells leads to suppression of a large number of non-neuronal transcripts (Lim et al., 2005) and a number of studies have shown that neuronal progenitor differentiation is the consequence of both de-repression of *REST* and downregulation of target mRNAs by miR-124a (Conaco et al., 2006; Wu et al., 2006). miR-124 was also shown to promote neuron differentiation by regulating the RNA-binding protein *PTBP1*, a global repressor of alternative pre-mRNA splicing in non-neuronal cells (Makeyev et al., 2007). More recently, Cheng et al. provided evidence that mir-124 regulates neurogenesis in the subventricular zone stem cell niche by repressing the SRY-box transcription factor *Sox9* in the adult mammalian brain (Cheng et al., 2009). Yu et al. showed that miR-124 controls neurite outgrowth in differentiating mouse P19 cells and mouse primary cortical neurons (Yu et al., 2008). In addition to its roles in neuronal development, Rajasethupathy et al. provided evidence that mir-124 also plays an important role in serotonin mediated long-term plasticity

of synapses in the mature nervous system of *Aplysia californica* (Rajasethupathy et al., 2009).

3. miRNA dysregulation in neuropsychiatric disorders

Evidence that miRNAs regulate many aspects of neural structure and function at multiple levels strongly suggests that alterations in miRNA regulation or function might contribute to the genetic and biological basis of neuropsychiatric disorders, as well as childhood intellectual disability and autism. A number of recent studies tested this hypothesis directly and provided supportive evidence, as summarized in detail below.

3.1 Schizophrenia

Schizophrenia (SCZ) is a devastating psychiatric disorder that has a lifetime prevalence of ~1% in most of the populations studied and is characterized by impaired cognition, positive psychotic symptoms such as hallucinations, delusions, and disorganized behavior, as well as negative symptoms such as social withdrawal and apathy. Epidemiological studies indicate a complex genetic component that interacts with epigenetic, stochastic, and environmental factors. Emerging evidence suggests that miRNAs might be involved in the etiology of SCZ. The strongest line of evidence for a direct pathogenic link between SCZ and miRNA biogenesis is provided by a recent study using a mouse model of the 22q11.2 microdeletion, one of the highest known risk factors for schizophrenia (Stark et al., 2008).

22q11.2 microdeletions account for up to 1–2% of non-familial (sporadic) SCZ cases and represent the only confirmed recurrent copy number variant (CNV, genomic gain or loss ≥ 1 -kb) responsible for introducing new cases of SCZ in the population (ISC, 2008; Karayiorgou et al., 1995; Stefansson et al., 2008; Xu et al., 2008a). It has been shown that ~30% of children with the 22q11.2 microdeletion will develop SCZ or schizoaffective disorder in adolescence or early adulthood (Murphy et al., 1999; Pulver et al., 1994). The most common deletion spans 3-Mb, but a nested 1.5-Mb deletion represents the SCZ critical region (Fig. 2) (Bearden et al., 2001; Kates et al., 2007; Lewandowski et al., 2007; Sobin et al., 2005). This 1.5-Mb human 22q11.2 locus is conserved within the syntenic region of mouse chromosome 16 and harbors nearly all orthologues of the human genes (Fig. 2). Stark et al. used a chromosomal engineering approach to generate a mouse model carrying the equivalent of the 1.5-Mb microdeletion (*Df(16)A^{+/-}*) (Stark et al., 2008). Using this mouse model, Stark et al. provided compelling evidence for an abnormality in brain miRNA processing in *Df(16)A^{+/-}* mice, emerging as a result of the hemizygous deletion of the *Dgcr8* gene, one of the disrupted 22q11.2 genes and an important component of the “microprocessor” complex (see section 2.1) (Stark et al., 2008). The end effect of *Dgcr8* haploinsufficiency is the down-regulation (by ~20–70%) of a specific subset of mature miRNAs, including mir-134 as well as other miRNAs within the same activity-regulated cluster (see section 2.2). It was estimated that impaired miRNA biogenesis could account for at least a portion of the transcript up-regulation observed in the prefrontal cortex and hippocampus of the *Df(16)A^{+/-}* mice. However, actual targets have not yet been reported and whether reduced miRNAs affect neural development and function by modulating protein output from many target genes or by impacting a few key proteins remains unclear (Stark et al., 2008).

To examine the functional consequences of alterations in miRNA biogenesis in *Df(16)A^{+/-}* mice and identify the miRNA regulated genes, Stark et al. generated *Dgcr8*-deficient mice and showed that haploinsufficiency at the *Dgcr8* locus and the ensuing alterations in miRNA biogenesis contribute to the behavioral and cognitive deficits observed in the *Df(16)A^{+/-}* mice. *Dgcr8*-deficient mice had impaired acquisition of a spatial working memory-dependent task, as well as impaired sensorimotor gating. By contrast, they had normal associative memory. This was the first time that abnormal miRNA biogenesis was shown to affect cognitive

performance in mice. As mentioned above, miRNAs may contribute to synaptic development and maturation providing, at least in part, a potential explanation for the cognitive and behavioral deficits observed in the *Dgcr8*^{+/-} mice. Indeed, *Dgcr8*-deficient mice demonstrated impaired dendritic tree and dendritic spine development (Stark et al., 2008).

Based on these findings, it was speculated that miRNA-dependent regulation of neuronal connectivity might contribute to the pathogenesis and pathophysiology of SCZ and other psychiatric disorders. Several recent lines of evidence, summarized below, appear to support this hypothesis and suggest that the potential of miRNAs to contribute to the regulation of expression of multiple genes in the brain could be an important component of the genetically complex architecture of such disorders.

First, human genetic studies appear to support a role of miRNAs in the genetically complex architecture of SCZ. For example, in a genome-wide scan for *de novo* CNVs in sporadic SCZ, Xu et al. identified a *de novo* duplication encompassing the *DICER1* gene (Xu et al., 2008a). Moreover, several large scale genome-wide scans for CNVs have identified a number of structural variants enriched in patients with SCZ, two of which, in addition to the 22q11.2 locus, contain miRNAs (Table 1). In addition, one of the candidate culprit genes within one of the recurrent CNV regions (15q11.2) is *CYFIP1*, which binds the Fragile X Mental Retardation Protein (FMRP, see section 5.5) and the translation initiation factor eIF4E (Napoli et al., 2008). Both FMRP and eIF4E have been implicated in miRNA-mediated translational control machinery (Jin et al., 2004; Pillai et al., 2004). Finally, a SNP-based genetic analysis identified a suggestive association of two miRNA loci, miR-206 and miR-198, with SCZ in a Danish and Norwegian sample (Hansen et al., 2007). Target prediction and pathway analysis suggested that 8 of the 15 potential target genes of both miR-198 and miR-206 are within a tight network including the transcriptional factors JUN, ATF2 and TAF1 (Beveridge et al., 2008).

Second, expression-profiling studies in postmortem brains of individuals with SCZ have indicated changes in miRNA levels. Perkins et al. conducted an expression profiling study of 264 human miRNAs using postmortem prefrontal cortex samples from patients with SCZ and unaffected controls. Fifteen miRNAs showed significant decreased expression and one showed higher expression in SCZ patients as compared with controls (Perkins et al., 2007). Another expression profiling analysis using postmortem cortical grey matter from the superior temporal gyrus revealed significant up-regulation of miR-181b expression in SCZ (Beveridge et al., 2009). The same group reported a significant SCZ-associated increase in global miRNA expression (both mature miRNAs and their precursor forms), as well as upregulation of the *DGCR8* mRNA in the superior temporal gyrus and the dorsolateral prefrontal cortex from postmortem SCZ patients. It is difficult to reconcile the Beveridge et al. (2009) study with the Perkins et al. (2007) study, as well as with the fact that only 22q11.2 microdeletions (one copy of *DGCR8*) but not 22q11.2 microduplications (three copies of *DGCR8*) predispose to SCZ (see also below). Additional studies are needed to resolve this discrepancy. Nevertheless, taken together all expression studies provide further support to the notion that altered miRNA levels could be a significant factor in the dysregulation of cortical gene expression in SCZ. Along these lines, Mellios et al. used a set of postmortem samples from the prefrontal cortex of 20 SCZ and 20 control subjects to test the association between BDNF protein, neuropeptide Y, somatostatin, and parvalbumin mRNAs, and two BDNF-regulating miRNAs (miR-195 and miR-30a-5p) (Mellios et al., 2009). They found that miR-195 changes in patient prefrontal cortex were inversely associated with those of BDNF protein. However, miR-195 levels remained unchanged in *Bdnf*-deficient mice. They suggested that miR-195 might operate as a fine-tuner of BDNF protein level, which in turn contributes to the prefrontal deficits in a subset of GABAergic mRNAs.

Finally, additional evidence for a role of miRNAs in psychiatric disorders comes from studies checking the impact of drugs (antipsychotics or psychotomimetics) on expression of miRNAs and related targets. Perkins et al. checked the expression levels of 179 miRNAs in rats treated with haloperidol as compared to untreated controls. Three miRNAs, mir-128a, mir-128b and mir-199a were up-regulated in the haloperidol-treated rats (Perkins et al., 2007). In another study, disruption of NMDA receptor signaling by dizocilpine, a selective NMDA receptor antagonist was shown to decrease miR-219 level in the prefrontal cortex of mice (Kocerha et al., 2009). *In vivo* inhibition of miR-219 by specific anti-miR in the murine brain caused up-regulation of its target calcium/calmodulin-dependent protein kinase II gamma subunit (CaMKII gamma). In turn, abnormal expression of CAMKII gamma resulted in malfunction of NMDA receptor signaling and alterations in relevant behavioral responses. Interestingly, the dizocilpine-induced effects on miR-219 could be attenuated by pretreating the mice with the antipsychotic drugs haloperidol and clozapine. In related studies employing drugs used for the management of bipolar disorder Zhou et al. screened for miRNA changes in hippocampi of rats chronically treated with either lithium or sodium valproate. Down-regulation of let-7b, let-7c, miR-128a, miR-24a, miR-30c, miR-34a, miR-221 and upregulation of miR-144 were confirmed by qPCR. Interestingly, one target of miR-34a is the metabotropic glutamate receptor gene GRM7, suggesting that mood stabilizers might upregulate GRM7 levels via miR-34a (Zhou et al., 2009).

3.2 Tourette's syndrome

Tourette's syndrome (TS), also called Gilles de la Tourette syndrome, is a neuropsychiatric disorder characterized by chronic vocal and motor tics and associated with behavioral abnormalities. The age of onset of the disease ranges from 2 to 14 years old with a peak age of tic onset at 6–7 years of age and its prevalence is estimated to be 1 in 100 individuals (Kereshian et al., 2009; Robertson, 2003). About 75% of TS patients are male (Staley et al., 1997). TS is often comorbid with other neuropsychiatric disorders, especially, attention deficit/hyperactivity disorder and obsessive-compulsive disorder (Cavanna et al., 2009). Genetic analysis including family, segregation and twin studies indicate that TS has strong genetic components. However, linkage and candidate gene association studies aimed to pinpoint causative genetic lesion have been largely unsuccessful (O'Rourke et al., 2009).

A connection between miRNA and TS was first proposed by Abelson et al. (2005). Based on a chromosomal breakpoint analysis of a *de novo* chromosomal inversion of a TS patient, Abelson et al. identified Slit and Trk-like1 (*SLITRK1*) gene, located close to the breakpoint, as a candidate gene for TS. Mutational screening of the *SLITRK1* gene in 174 unrelated TS patients revealed that two patients (but none of the 2,148 controls) carried a sequence variant (var321) in the 3'UTR of *SLITRK1*, which affects the binding of a miRNA, hsa-mir-189. It was shown that mir-189 has a modest dose-dependent effect on *SLITRK1* expression in a luciferase assay system. By using *in situ* hybridization on postnatal mouse and fetal human brains, Abelson et al. showed that the expression of mir-189 and *SLITRK1* mRNA are overlapping in many neuroanatomical circuits that are most commonly implicated in TS (Abelson et al., 2005). Finally, it was shown that over-expression of *SLITRK1* in cortical neurons in culture can promote dendritic growth (Abelson et al., 2005).

This work provided a possible mechanism of how miRNAs might be involved in TS etiology. Several follow-up mutational screens were conducted in additional samples (Chou et al., 2007; Deng et al., 2006; Keen-Kim et al., 2006; Scharf et al., 2008; Verkerk et al., 2006; Wendland et al., 2006; Zimprich et al., 2008). The var321 variant has been detected in some of these studies, but failed to segregate with TS. Given that var321 has a very low allele frequency (~0.1% in the general population) one likely explanation for these discrepancies could be that replication studies might have been underpowered although the possibility of a

false positive original finding (i.e. due to population stratification, (Keen-Kim et al., 2006) cannot be excluded. Additional studies with even larger samples in homogeneous populations are needed to clarify the role of var321 and hsa-mir-189 in TS susceptibility.

3.3 Autism spectrum disorders

Autism spectrum disorders (ASDs) are a heterogeneous group of neuropsychiatric disorders with core clinical symptoms specified by DSM-IV as qualitative impairment in social interaction plus repetitive and stereotyped behaviors (DSM-IV, American Psychiatric Association, 1994). The prevalence of ASDs is 1% and mainly affects males with an onset 3 years of age or earlier.

ASDs have a strong genetic component as estimated by family and twins studies (Bailey et al., 1995; Folstein et al., 1977; Greenberg et al., 2001; Steffenburg et al., 1989) and siblings of affected individuals have a much higher disease risk than individuals in the general population (Muhle et al., 2004). Despite the evidence that ASDs are influenced heavily by genetics, it has been extremely difficult to pinpoint the culprit genetic variants. However, similar to the situation in SCZ described above, a number of recent studies indicated that rare CNVs collectively contribute to ASD risk (Bucan et al., 2009; Cho et al., 2009; Christian et al., 2008; Glessner et al., 2009; Kumar et al., 2008; Marshall et al., 2008; Miller et al., 2009; Sebat et al., 2007; Szatmari et al., 2007; Weiss et al., 2008).

Several recent studies started to investigate whether dysregulation of miRNAs also plays a role in ASDs. Talebizadeh et al. examined the expression profile of mature human miRNAs using lymphoblastoid cell line samples. The authors screened 470 human miRNAs (miRBase Sequence database version 9.0) in 6 ASD patients and 6 matched controls and identified nine miRNAs that showed differential expression in the ASD samples as compared to controls (Talebizadeh et al., 2008). In an independent study, Abu-Elneel et al. used a multiplex qPCR approach to screen the expression of 466 human miRNAs in the postmortem cerebellar cortex of 13 individuals with ASDs and 13 non-autistic controls. The authors showed that 28 of 277 reliably detected miRNAs were differentially expressed in at least one of the autism samples as compared to the mean value of non-autism controls (Abu-Elneel et al., 2008). None of these dysregulated miRNA loci overlapped with genomic regions previously associated with ASDs by linkage or association studies.

A potential connection between miRNAs and ASD-associated CNVs was also established directly through human genetic studies. Notably, a number of genome-wide assays for CNVs identified a significant enrichment of 22q11.2 microduplications in unrelated ASD cases (Glessner et al., 2009; Marshall et al., 2008). By contrast, no such enrichment of 22q11.2 microduplications has been found in SCZ (Brunet et al., 2008). Microduplication of the 22q11.2 locus results in an increase in the dosage and expression of the *DGCR8* gene and likely affects miRNA biogenesis (BX, MK, JAG unpublished). In addition to the 22q11.2 duplication, a recurrent genomic imbalance at 15q13.2-q13.3 region, which contains hsa-mir-211, was also associated with ASDs, intellectual disability, epilepsy, and/or electroencephalogram (EEG) abnormalities (Miller et al., 2009) (Table 1). The degree of overlap between miRNA loci and autism associated CNVs remains unknown but it may extend beyond the two examples mentioned above. Taken together these results suggest that miRNA alterations could contribute to the genetic heterogeneity and phenotypic variation of ASDs.

3.4 Rett syndrome

Rett syndrome (RTT) is an X-linked neurodevelopmental disorder occurring primarily in females with an incidence of 1:10,000–15,000 (Hagberg, 1985). Patients with classic RTT have an apparently normal development before 6–18 months of age, then gradually exhibit

developmental stagnation, stereotypical movements, microcephaly, seizures, autistic features and intellectual disability (Hagberg et al., 1983). Epileptic seizures are reported in up to 90% of girls with RTT (Witt Engerstrom, 1992). The major morphological changes in the brains of RTT patients are decrease in the size of the brain as well as decrease in the size and dendritic arborization of individual neurons (Armstrong et al., 1995; Leonard et al., 1998; Sirianni et al., 1998). Ninety-nine percent of affected girls are sporadic cases. Nevertheless, because of its dominant X-linked inheritance pattern, exclusion mapping studies using RTT families were able to map the lesion locus to Xq28 (Archidiacono et al., 1991; Curtis et al., 1993; Ellison et al., 1992; Sirianni et al., 1998; Witt Engerstrom, 1992) and a systematic gene screening finally determined that mutations in the gene encoding methyl-CpG binding protein 2 (*MeCP2*) are the main cause of RTT (Amir et al., 1999).

Evidence that miRNAs are involved in the etiology and clinical expression of RTT was originally provided by studies examining how miR-132, a brain enriched miRNA, can contribute to BDNF-mediated neurite outgrowth of neonatal neurons. Klein et al. discovered that a long 3'UTR isoform of MeCP2 is under the control of miR-132 in primary cortical neurons and this regulation is both necessary and sufficient in controlling MeCP2 protein levels. This finding, combined with the observations that BDNF induces miR-132 transcription and that lack of MeCP2 decreases BDNF levels in mouse models of RTT, suggests that miR-132 might exert homeostatic control over MeCP2 translation (Klein et al., 2007; Vo et al., 2005). Another study reported that MeCP2 can bind to the promoter region of another brain-specific miRNA, miR-184, and repress its expression (Nomura et al., 2008). It was further shown that, upon depolarization, MeCP2 was released from the promoter binding site of the paternal allele and led to up-regulation of paternal allele-specific expression of miR-184 in cultured mouse cortical neurons. However, the authors failed to observe up-regulation of mir-184 in the *Mecp2*-deficient mouse brain and instead, they observed a decrease of mir-184 levels. In addition, no morphological changes were identified when mir-184 was over-expressed in the cultured cortical neurons (Nomura et al., 2008). Additional studies are needed to clarify the relationship between *Mecp2* and mir-184.

3.5 Fragile X syndrome (FXS)

FXS is the most common form of inherited intellectual disability, affecting 1:4000 males and about half as many females (Turner et al., 1996). In addition to cognitive and behavioral impairments (Tsiouris et al., 2004), speech and language skills are also often affected in male patients as seen in ASD patients (Merenstein et al., 1996). FXS is caused by mutations in the Fragile X mental Retardation protein (*FMRP*) gene within the fragile X syndrome breakpoint cluster region on Xq27–28 (Devys et al., 1993; Verkerk et al., 1991). Most patients display a trinucleotide (CGG) repeat expansion of more than 200 repeats in the 5'-UTR of *FMRP* causing hypermethylation and silencing of FMRP (Penagarikano et al., 2007). FMRP is a RNA binding protein and is thought to act as a neuronal translational repressor. Mutations in FMRP affect neuron morphology, synaptic plasticity and long term memory in patients and animal models (Bolduc et al., 2008; Dichtenberg et al., 2008; Huber et al., 2002; Jin et al., 2004; Zhang et al., 2001).

The initial evidence that FMRP may be associated with the miRNA pathway came from the identification of FMRP association with Argonaute-2 (Ago2) and Dicer in both flies and mammals (Caudy et al., 2002; Ishizuka et al., 2002; Jin et al., 2004). In addition, Xu et al. showed that miR-124a (see section 2.2) is associated with the *Drosophila* homolog of FMRP (dFMR1) *in vivo* (Xu et al., 2008b). Ectopic expression of miR-124a precursors decreased dendritic branching of sensory neurons. This effect could be partially rescued by the loss of dFMR1 activity, suggesting that the biogenesis and/or function of miR-124a are partially dependent on dFMR1. Indeed, the steady-state levels of endogenous or ectopically expressed

mature miR-124a were also partially reduced in dFMR1 mutants whereas pre-miR-124a levels were increased (in contrast to the complete loss of mature miR-124a in Dicer-1 mutants). Reduced abundance of mature miR-124a could be caused by the reduction of the Dicer-1-Ago1 complex in the absence of dFMR1. These findings suggest a modulatory role for dFMR1 in maintaining proper levels of miRNAs during neuronal development (Xu et al., 2008b). More recently, Edbauer et al. identified several FMRP-association miRNAs from mouse brain including mir-125b, as well as mir-132 (see section 3.4) (Edbauer et al., 2010). They further showed that alterations of mir-125b expression resulted in spine morphology changes and confirmed the role of mir-132 on dendritic spine morphology as previously reported (Vo et al., 2005; Wayman et al., 2008). Interestingly, it was shown that the effect of mir-125b and mir-132 on the morphology of mouse hippocampal neurons is FMRP-dependent and was abolished in cells with FMRP knockdown. Furthermore, it was shown that the expression of the NMDA receptor subunit NR2A is regulated by FMRP partially through mir-125b (Edbauer et al., 2010). This result is consistent with previous observations that loss of FMRP modulates NMDA receptor function in mice (Pfeiffer et al., 2007; Pilpel et al., 2009).

3.6 Down syndrome

Down syndrome (DS), also called Trisomy 21, is a chromosomal disorder in which all or part of an extra chromosome 21 causes mild to moderate intellectual disability in addition to other health problems, including precocious dementia, heart defects, early-onset Alzheimer's disease and childhood leukemia. The prevalence of DS is 1 in 800–1,000 births (Carothers et al., 1999). The extra chromosome 21 has a significant impact on nervous system development. The brain of DS patients shows impaired maturation, has atrophic dendritic structure, decreased neuronal numbers, abnormal neuronal differentiation and early appearance of senile plaques (Mrak et al., 2004; Wisniewski et al., 1985).

Recently, Kuhn et al. used microarrays, RT-PCR and miRNA *in situ* hybridization methods to investigate five miRNA genes (miR-99a, let-7c, miR-125b-2, miR-155, and miR-802) located on human chromosome 21. They showed that these miRNAs were up-regulated in the fetal brain tissue of DS patients compared to age- and sex-matched controls (Kuhn et al., 2008). In a follow-up study, the same group used luciferase/target mRNA 3'UTR reporter assays to demonstrate that miR-155 and miR-802 can regulate MeCP2 expression. In DS brain samples from both humans and mouse models, MeCP2 was down-regulated and its downstream targets, Creb1 and Mef2c, were also altered. When endogenous miR-155 or miR-802 were knocked-down by intra-ventricular injections of antagomirs, the expression of MeCP2 and its targets returned to normal levels (Kuhn et al., 2009). These results suggest that over-expression of the miRNAs on chromosome 21 may cause improper repression of MeCP2, which in turn contributes, in part, to the neural deficits observed in the brains of DS individuals. It is worth noting that in search of genes responsible for the DS phenotype, a Down syndrome critical region was previously identified to be associated with many of the DS phenotypes, especially intellectual disability (Delabar et al., 1993). However, none of these miRNAs are located within the Down syndrome critical region and, therefore, connections between these miRNAs and intellectual disability requires further exploration.

4. Conclusions

The evidence presented above strongly suggests that miRNAs play an important role in the pathogenesis and pathophysiology of neuropsychiatric and neurodevelopmental disorders. In general, miRNAs appear to be components of both the genetic architecture of these complex phenotypes as well as integral parts of the biological pathways that mediate the effects of primary genetic deficits. Therefore, they may contribute to both genetic heterogeneity and phenotypic variation. However, for the most part, their exact mode of action remains unclear

and, very likely, will be the subject of intense scrutiny in the near future. Eventually our understanding will improve by the convergence of findings from both basic and disease-oriented research as well as from the development of reliable animal models. A comprehensive understanding of the roles of miRNAs in CNS will be important for determining whether miRNAs-related pathways could serve as novel targets for drug development for a number of neuropsychiatric and neurodevelopmental diseases.

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Figure 1. Components of miRNA biogenesis and mature miRNAs implicated in neural structure and function as well as in neuropsychiatric disorders and cognitive dysfunction
 SCZ = schizophrenia, ASD = Autism spectrum disorder; FXS =Fragile X syndrome; RTT = Rett syndrome; DS = Down syndrome; TS = Tourette's syndrome

Figure 2. Genomic structure of the human 1.5-Mb 22q11.2 locus and the syntenic mouse region and consequences of *DGCR8* haploinsufficiency

Schematic diagram of the genomic structure of the 1.5-Mb human 22q11.2 microdeletion and the syntenic mouse region on chromosome 16. Almost all of the functional human genes in this segment are conserved in the mouse, organized in a slightly different order. The 1.5-Mb deletion is mediated by low copy repeat sequences LCR-A and LCR-B (illustrated as red boxes). *PRODH-P* and *DGCR6-like* indicate pseudogenes. *Dgcr2* and *Hira*, the two endpoints of the targeted deletion of the mouse locus, are indicated by red arrowheads. The effect of *DGCR8* haploinsufficiency on miRNA levels, CA1 pyramidal neuron dendritic morphology as well as behavior is summarized at the bottom. Hemizygosity of the *Dgcr8* gene results in

reduction in the levels of a subset of mature miRNAs in both prefrontal cortex and hippocampus. Partial reduction in miRNA levels can have an effect on transcript or protein levels of target genes. Moreover, since target mRNAs can be simultaneously and synergistically bound and repressed by more than one miRNA, the level of repression achieved may be highly sensitive to the amount of available miRNA complexes and the number of affected miRNAs. miRNA dysregulation is one of the molecular pathways affected in 22q11.2DS, is predicted to change the levels of several downstream target transcripts and proteins and results in a number of phenotypic alterations.

Table 1

miRNAs within recurrent CNV's identified in SCZ and ASD

chr	start	end	Reported by	Disease	miRNAs within the region
15	28720000	30300000	ISC 2008;Stefansson et al., 2008; Kirov et al., 2008; Kirov et al., 2009; Miller et al., 2008	SCZ, ASD	hsa-mir-211
16	15387381	18072544	Kirov et al., 2009; Ingason et al., 2009	SCZ	hsa-mir-484
22	17052138	18585938	Karayorgou et al., 1995; Xu et al., 2008; ISC, 2008; Stefansson et al., 2008	SCZ (deletion), ASD (duplication)	hsa-mir-185, hsa-mir-1306
22	16926763	19926763	Karayorgou et al., 1995; Xu et al., 2008; ISC, 2008; Stefansson et al., 2008	SCZ (deletion), ASD (duplication)	hsa-mir-185, hsa-mir-1306, hsa-mir-1286, hsa-mir-649