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miRNA GENES AND THE BRAIN: IMPLICATIONS FOR PSYCHIATRIC DISORDERS

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ABSTRACT

MicroRNAs (miRNAs) are a class of non-protein coding genes with a growing importance in regulatory mechanisms of gene expression related to brain function and plasticity. Considering the relative lack of success of the analysis of variations in candidate protein coding genes and of genome-wide association studies to identify strong risk factors for common psychiatric disorders (PDs), miRNA genes are of particular interest for the field of psychiatric genetics as deregulation of the rate of transcription or translation of a normal gene may be phenotypically similar to disruption of the gene itself. In this paper, we review the current knowledge on the contribution of miRNAs in basic mechanisms of brain development and plasticity and their possible involvement in the pathogenesis of several PDs. Since future functional and genomic explorations of brain expressed miRNAs, and other types of non-coding RNAs, may identify additional candidate genes and pathways for common PDs, we believe that implementing additional strategies to further elucidate the role of miRNAs in the etiology of common PDs is of great importance.

KEYWORDS: Brain disorders, psychiatric diseases, schizophrenia, microRNAs, non-coding RNA

1. INTRODUCTION

Molecular genetics of psychiatric disorders

Psychiatric disorders (PDs) are debilitating diseases characterized by the dysfunction of complex cognitive and emotional processes and the presence of multiple behavioural symptoms. PDs have been classified in different categories depending on the clustering of their associated symptoms: mood disorders (bipolar disorder -BP- and major depressive disorder -MDD-), psychotic disorders (schizophrenia -SZ-), anxiety disorders (panic disorder and generalized anxiety disorder); pervasive developmental disorders (autism spectrum disorders -ASD-), among others (Kendler, 2006). As five out of ten leading causes of disability in the world are related to PDs (being MDD, Alcohol use, BP, SZ and obsessive-compulsive disorders) it is obvious that PDs present a large burden on society (Ramocki and Zoghbi, 2008).

Multiple lines of evidence show that some PDs have a strong genetic basis. For example, the heritability for BP, SZ and Attention Deficit Hyperactivity Disorder -ADHD- is estimated to be 0.59, 0.64 and 0.76, respectively (Lichtenstein et al., 2009; Faraone et al., 2005). In addition, there is evidence suggesting shared genetic susceptibility for some of these PDs, such as between BP and SZ (Lichtenstein et al., 2009). Despite this reported high heritability, joint efforts for the identification of susceptibility genes for PDs have shown little success. Meta-analyses of genome-wide linkage studies (Ng et al., 2009; McQueen et al., 2005) have identified significant signals in several large genomic regions containing thousands of genes. However, the specific underlying genetic alterations in those regions remain to be identified (Burmeister et al., 2008). Even the recent introduction of high density SNP arrays enabling genome wide association studies (GWAS) in thousands of patients and control individuals, has failed to identify strong and consistent risk factors for common PDs. There are published reports of 8 GWAS for BP and 10 GWAS for SZ (Alaerts and Del-Favero, 2009). In addition, there are several GWAS reports for MDD (Muglia et al., 2008; Sullivan et al., 2009), ADHD (Neale et al., 2008; Lesch et al., 2008) and ASD (Ma et al., 2009; Wang et al., 2009) with the top SNPs in these studies having low odd ratios (ORs) with the lower confidence intervals very close to 1 (Scott et al., 2009; Stefansson et

1 al., 2009). A detailed analysis of the top results from these GWAS for major PDs reveals that
2 there is little consistency between studies. In the case of BP and SZ, only 5 (out of 174) and 5 (out
3 of 194) genes had support from more than one GWAS, respectively (Supp. Tables S1 and S2). For
4 MDD, ADHD and ASD the general picture was even worse, with not a single gene being
5 supported from more than one study (Supp. Table S3). Even the most recent studies carrying out
6 meta-analyses of GWAS in tens of thousands of patients and controls do not provide strong
7 candidate genes for PDs.

8 Because of these apparent inconsistencies in genetic studies the complexity of PDs are assumed to
9 be caused by genetic heterogeneity, where defects in multiple risk genes – either in combination
10 or not – cause the same disorder. Apart from this ‘locus heterogeneity model’, another explanation
11 is that not the coding DNA of PD risk genes is disrupted but their regulation (Perkins et al., 2005).
12 The fact that only a minor part of the top ~1,500 GWAS SNPs were located in protein coding
13 regions (2.1% located in exons, 1.5% being nsSNPs) supports this hypothesis. The majority of
14 identified SNPs were located in intergenic regions (55.1%), with 7.2% of the SNPs located in
15 conserved regions in vertebrates. In addition, 4% of the top SNPs were located in UTR regions
16 (Supp. Table S4). Therefore, the exploration of additional categories of non-coding genes and
17 molecules is a potentially interesting approach for the identification of genetic factors implicated
18 in PDs.

19 In recent years, microRNAs (miRNAs) have emerged as a new important class of non-coding
20 RNA (ncRNA) regulatory molecules, playing a role in many major cellular processes related to
21 brain function (Fiore et al., 2008). Because deregulation of the rate of transcription and/or
22 translation of a normal gene may be phenotypically similar to disruption of the gene itself, the
23 exploration of these ncRNA molecules might provide candidate genes for common PDs and give
24 additional clues about the possible role of miRNA-dependent pathways in the pathogenesis of
25 PDs (Perkins et al., 2005). In this review we provide an overview of current knowledge on the
26 contribution of miRNAs in basic mechanisms of brain development and plasticity and their
27 possible involvement in the pathogenesis of several PDs.

miRNA processing and function

The first papers describing miRNAs in humans appeared in 2000-2001 (Lagos-Quintana et al., 2001; Pasquinelli et al., 2000). Currently, there are already 706 known miRNAs for humans (miRBase release 13.0; 03/2009) (Griffiths-Jones et al., 2008). These miRNA genes can be divided in two main categories based on their location and transcription patterns: intronic miRNAs and intergenic miRNAs (Bartel, 2009). RNA polymerase II transcribes a major part of miRNAs (Lee et al., 2004) and the resulting primary miRNAs (pri-miRNAs) are similar to classical mRNAs, being several kb long, polyadenylated and regulated by networks of transcription factors (Cai et al., 2004; Lee et al., 2004; Saini et al., 2007) (Figure 1).

After the pri-miRNAs are transcribed, they are processed by the Microprocessor complex, composed of the Rnase III enzyme Drosha and Pasha (also known as DGCR8) (Denli et al., 2004; Gregory et al., 2004) into ~70 nucleotide precursor-miRNA (pre-miRNA) which are exported to the cytoplasm by Exportin 5 (Lund et al., 2004) (Figure 1). In the cytoplasm, Dicer cleaves pre-miRNAs in a transient double-stranded RNA fragment of about 18-24 nucleotides. The duplex is loaded into the miRNA associated multiprotein RNA-induced silencing complex (RISC), composed of Argonaute proteins (Liu et al., 2004; Diederichs and Haber, 2007) and other recently described molecules (Gregory et al., 2005), such as TARBP2, EIF6, FXR1 and MOV10 (Chendrimada et al., 2007; Liu et al., 2007; Macrae et al., 2008; Jin et al., 2004) (Supp. Table S1). One strand of the miRNA is preferentially retained in this complex and becomes the mature miRNA while the opposite strand, known as the passenger strand is eliminated from the complex.

It has been demonstrated that a 5' region in the miRNA, called the "seed", is the most important part, in terms of binding to the 3'UTR region of the target genes (Bartel, 2009). There are 3 main types of binding profiles: canonical sites (binding of only the seed region), 3' supplementary sites (binding of the 3' region of the miRNA, in addition to the seed region) and 3' compensatory sites (binding of the 3' region of the miRNA with presence of mismatches in the seed region) (Bartel, 2009). In animals the main consequence of miRNA targeting appears to be

1 the deadenylation of the mRNA leading to its instability (Forstemann et al., 2007; Karginov et al.,
2 2007) and/or the repression of RNA translation (Filipowicz et al., 2008; Pillai et al., 2005).
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8 2. miRNAS AND THE CENTRAL NERVOUS SYSTEM

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10 miRNAs regulate the expression of over 10,000 genes in a cell. It is therefore not surprising that
11 miRNAs are involved in the regulation of almost all major cellular functions, such as cell
12 differentiation, proliferation/growth, mobility, and apoptosis (Zhang et al., 2010). In recent years,
13 the interest in miRNAs and their possible roles in regulatory molecular mechanisms underlying
14 the functional and structural complexity of the brain have grown rapidly (Fiore et al., 2008)
15 (Table 1). The identification of brain-derived miRNAs has provided an initial catalogue of
16 miRNAs of potential relevance for brain function (Kim et al., 2004; Miska et al., 2004; Wheeler et
17 al., 2006; He et al., 2007). Moreover, more systematic analysis of miRNA expression in brain
18 tissues in different developmental stages in humans and rodents have been useful to identify those
19 miRNAs that are brain-specific (expressed only in brain) or brain-enriched (expressed at higher
20 levels in brain than in other tissues) (Bak et al., 2008; Kim et al., 2004; Liang et al., 2007; Miska
21 et al., 2004; Olsen et al., 2009; Zhao et al., 2006) (Supp. Table S2 and supp. Figure S2).
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39 miRNAs are believed to play a critical role in brain development and structural plasticity.
40 Evidence for this comes from the identification of the CREB (cAMP response element binding)
41 and BDNF (brain derived neurotrophic factor) dependent activation of miR-132 and miR-219 (Vo
42 et al., 2005; Cheng et al., 2007; Klein et al., 2007; Wayman et al., 2008). Other studies have
43 provided evidence supporting a role of proteins involved in miRNA processing (such as Dicer,
44 DGCR8, FXR1 or MOV10) in mechanisms of brain plasticity (Xu et al., 2004; Lin et al., 2006;
45 Karr et al., 2009; Jin et al., 2004; Giraldez et al., 2005; Davis et al., 2008; Ashraf et al., 2006).
46 Down regulation of Dicer led to molecular, morphological and physiological alterations in brains
47 of zebrafish, fly and mouse models (Giraldez et al., 2005) (Davis et al., 2008) (Karr et al., 2009).
48 Mutations in *Drosophila dfmr1* (homolog of the human FXR1 gene) and *armitage* (homolog of
49 the human MOV10 gene) led to structural brain changes and behavioural abnormalities (Xu et al.,
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2004; Lin et al., 2006; Jin et al., 2004; Ashraf et al., 2006). Knockout mice for Dgcr8 presented structural brain changes and behavioural alterations (Stark et al., 2008). miRNAs have also been implicated in mechanisms underlying different behavioural paradigms in animal models (e.g. circadian rhythms, olfactory memory or sleep deprivation) (Cheng et al., 2007; Ashraf et al., 2006; Davis et al., 2007). In mice, for instance, antagomirs (i.e. small synthetic RNA molecule perfectly complementary to the miRNA target with either mispairing at the Ago2 cleavage site or base modification to inhibit Ago2 cleavage) against miR-219 and miR-132 regulated the circadian period length and resetting of the clock, respectively (Cheng et al., 2007).

3. miRNAS AND PSYCHIATRIC DISORDERS

The data summarized in the previous section demonstrate how alterations of specific brain-expressed miRNAs may lead to altered expression levels of many genes involved in mechanisms of neural and synaptic plasticity, which are associated with the pathophysiology of several major neuropsychiatric disorders (Forero et al., 2006; Ramocki and Zoghbi, 2008). Although genome-wide expression and proteomics experiments in post-mortem brains of patients with major PDs have not provided protein coding genes with consistent deregulation between studies (Choi et al., 2008; Elashoff et al., 2007; Higgs et al., 2006), there is evidence that several signalling networks related to synaptic and brain plasticity are altered in these disorders (Ramocki and Zoghbi, 2008; Carter, 2006; D'Sa and Duman, 2002; Mudge et al., 2008; Ryan et al., 2006; Schloesser et al., 2008). These findings, in addition to expanding the repertoire of molecules and basic mechanisms related to brain plasticity; provide useful insights to explore potential roles of miRNA-dependent mechanisms in the pathogenesis of PDs. An overview of miRNA-regulated signalling networks important for brain plasticity and involved in PDs is shown in Figure 2.

Thus far, only a limited number of studies have addressed the role of miRNA dysfunction in PDs (Hunsberger et al., 2009). Systematic expression studies of post-mortem brains of psychiatric patients and of animal models have provided initial insights about specific miRNAs potentially involved in the pathogenesis of PDs (Hunsberger et al., 2009). In addition, exploration of common

1 and rare variants in miRNA genes and in their binding sites has given additional candidate genetic
2 factors associated with several PDs. In the following paragraphs we present an overview of recent
3 studies that have examined the biological role of miRNAs in PDs.
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8 Considering the difficulties of getting access to brain tissues of patients, cell based models
9 (especially lymphoblasts) and animal models of PDs (both genetic and pharmacological) are
10 potential approaches to study of miRNA alterations in psychiatric disorders. An overview of
11 miRNAs known to be deregulated in tissues of patients and/or animal and cell models for PDs
12 (SZ, BP, ASD, mood disorders and drug abuse) (Abu-Elneel et al., 2008; Beveridge et al., 2009;
13 Chandrasekar and Dreyer, 2009; Chen et al., 2009; Kocerha et al., 2009; Meerson et al., 2009;
14 Mellios et al., 2008; Perkins et al., 2005; Stark et al., 2008; Talebizadeh et al., 2008; Uchida et al.,
15 2008; Zhou et al., 2009; Zhu et al., 2009) is shown in Table 2.
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27 Thus far, three miRNA expression studies in post-mortem brains of SZ patients have been
28 performed, which have led to several interesting insights (Beveridge et al., 2008; Perkins et al.,
29 2007; Beveridge et al., 2009). In a first study, Perkins et al. (2007) used prefrontal cortex tissue of
30 13 schizophrenia patients and 21 controls to analyze the expression of 265 mature miRNAs. 16
31 miRNAs were differentially expressed (15 down-regulated, 1 up-regulated) and 4 out of 8 down-
32 regulated miRNAs were confirmed by RT-PCR. Several signalling pathways related to
33 mechanisms of synaptic function, such as calcium-dependent gene regulation, were enriched in
34 the predicted target genes of these deregulated miRNAs (Perkins et al., 2007). Beveridge et al.
35 (2008) used RNA isolated from the superior temporal gyrus of 7 SZ-control pairs to study 262
36 miRNAs and they confirmed the up-regulation of two miRNAs. The over expression of one of
37 these miRNAs – miR-181b – was also shown by northern blot and RT-PCR. Here also, functional
38 categories related to synaptic function, such as axon guidance, were enriched among the predicted
39 targets of miR-181b (Beveridge et al., 2008). The same group expanded their results and studied
40 miRNA expression in a larger group of 17 SZ/control pairs for superior temporal gyrus and 15
41 SZ/control pairs for dorsolateral prefrontal cortex. In patients, 21 and 9.5 percent, respectively, of
42 the expressed miRNAs in those tissues were up regulated. Members of the miR-15 family were
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1 particularly prominent among these up-regulated miRNAs. Again, deregulation of signalling
2 pathways related to mechanisms of synaptic function was implicated, as shown by the enrichment
3 of predicted target genes of the miR-15 family in these functional categories. Because these
4 increased pre-miRNA expression levels were accompanied by normal values for pri-miRNAs
5 expression levels, the authors suggested a global alteration in miRNA processing in SZ brains
6 which was correlated with increased mRNA levels for Drosha, DGCR8 and DICER1. Four
7 predicted targets of the miR-15 family of relevance for psychiatric genetics were experimentally
8 validated (RGS4, GRM7, GRIN3A and RELN) (Beveridge et al., 2009).

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10 Further evidence supporting a role for miRNA alterations in PDs comes from the use of genetic
11 and pharmacological animal models. Stark et al. (2008) generated a mouse model with a
12 hemizygous 1.3 Mb deficiency of a genomic region that is syntenic to the 1.5 Mb region missing
13 in the 22q11.2 microdeletion found in SZ patients; it contains 27 genes, including Dgcr8. These
14 animals showed behavioural alterations in several domains of relevance for PDs: locomotor
15 hyperactivity, anxiety-like behaviours and deficits in prepulse inhibition, cued and contextual fear
16 conditioning and working memory. Microarray analysis of hippocampus and prefrontal cortex of
17 these animals showed alterations in the expression levels of multiple genes related to synaptic
18 function. Expression levels of Dgcr8 were reduced, while no differences were seen in the
19 expression levels of other genes involved in the miRNA processing machinery (Rnasen, Dicer1,
20 among others). When further investigating the effect of this down regulation on miRNA
21 processing, levels of pri-miRNAs were increased while mature miRNAs had decreased levels,
22 which was also confirmed in heterozygous knockout mice for Dgcr8. This study is one of the first
23 to suggest a potential link between a genetic alteration found in SZ patients (22q11.2
24 microdeletion) and a broad dysfunction in miRNA-dependent mechanisms of transcriptional
25 regulation mediated by alterations in a gene involved in miRNA processing (DGCR8) (Stark et
26 al., 2008).

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28 Zhou et al. (2009) treated rats with lithium and valproic acid and found respectively 37 and 31
29 responsive miRNAs with 9 miRNAs in common. GRM7, a glutamate metabotropic receptor and
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1 one of the targets of one of these miRNAs (miR-34a) had increased protein levels in hippocampal
2 regions of the treated animals (Zhou et al., 2009). In another study, mice were treated with a
3 selective phencyclidine-like NMDA antagonist (dizolcipine) and found that miR-219 was down-
4 regulated in the prefrontal cortex (Kocerha et al., 2009). Possible involvement of this miR-219
5 gene in PDs was suggested by the observation that this dizolcipine-induced down-regulation could
6 be prevented by the treatment with antipsychotics (haloperidol and clozapine) and the use of an
7 antagomir against miR-219 reduced the behavioural effects of dizolcipine treatment.
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20 Although miRNA genes are usually highly conserved through evolution, there is evidence of intra
21 individual variation in some of this conserved non-protein coding regions in the human genome
22 (Drake et al., 2005; Quach et al., 2009; Adams et al., 2007; Duan et al., 2007; Muinos-Gimeno et
23 al., 2009b; Saunders et al., 2007). Variations in candidate miRNA genes or in their binding sites
24 have been analyzed in several PDs, such as Tourette syndrome (Abelson et al., 2005),
25 schizophrenia (Burmistrova et al., 2007; Hansen et al., 2007; Feng et al., 2009), mental retardation
26 (Chen et al., 2007; Zhang et al., 2006), conduct disorder-related behaviours (Jensen et al., 2009;
27 Conner et al., 2009), nicotine dependence (Huang and Li, 2009), panic disorder (Muinos-Gimeno
28 et al., 2009a) and obsessive compulsive disorder (Muinos-Gimeno et al., 2009a). An overview of
29 published genetic variations in PD associated miRNA genes and 3'UTR miRNA binding sites is
30 provided in tables 3 and 4.
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46 As an example, Chen et al. (2007) screened brain-expressed miRNA genes located on
47 chromosome X in 464 patients with X linked non-syndromic mental retardation and found 3
48 patient-specific variants (two in pre-miR-222 and one in pre-miR-363, each being present in only
49 one patient). The two variations in miR-222 co-segregated with the affection status (Chen et al.,
50 2007).
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58 A few studies uncovered genetic variations in miRNA binding sites. Abelson et al (Abelson et al.,
59 2005) identified a variation in the binding site for miR-189 in the 3' UTR region of *SLITRK1* in 2
60 unrelated patients (with Tourette syndrome and obsessive compulsive symptoms) not found in

1 2148 control subjects. Muinos-Gimeno et al (Muinos-Gimeno et al., 2009a) sequenced the 3'
2 UTR region of *NTRK3* (a neurotrophin receptor) and identified two variants in 212 Spanish
3 patients with panic disorder which led to allele-dependent effects on gene expression.
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10 **4. OTHER ncRNAs AND THE BRAIN**

11 It is evident that miRNAs are part of a bigger picture of ncRNA dependent regulation of gene
12 expression (Kapranov et al., 2007; Rigoutsos et al., 2006). Unfortunately, few studies have
13 performed to elucidate the role of other types of ncRNAs, such as long ncRNAs, on brain
14 function. A comprehensive analysis of the transcriptional landscape in mice (FANTOM3 project)
15 identified a total number of 181.047 independent transcripts, from which 34.030 corresponded to
16 potential long ncRNAs (Carninci et al., 2005). These long ncRNAs are suspected to be involved
17 in the regulation of several target genes, possibly through anti-sense based mechanisms
18 (Katayama et al., 2005). Mercer et al. (2009) studied the expression of long ncRNAs in different
19 mouse brain regions using high-throughput RNA in situ hybridization and found 849 long
20 ncRNAs with expression levels above background. Of these 239 have conserved secondary
21 structures; 60 showed ubiquitous expression and 513 showed enriched expression profiles in
22 specific regions (Mercer et al., 2008). Another microarray based study by Mercer et al. (2010) for
23 systematic analysis of expression profiles of long ncRNAs during differentiation of mice
24 embryonic forebrain derived neural stem cells showed several ncRNA expression patterns
25 associated with the differentiation mechanisms from stem cells to neural or glial cell types. In
26 addition, they showed that brain-expressed long ncRNAs are preferentially located in genomic
27 regions close to protein coding genes involved in brain development and function indicating
28 spatiotemporal co-expression. A specific long ncRNA (*Evf2*) was shown to modulate the
29 expression of several transcription factors (such as *MECP2* and *DLX5*) in developing neurons of
30 mice (Bond et al., 2009). *Evf2* mutants showed reduced numbers of GABAergic interneurons
31 during brain development. Sone et al (2007) identified a novel ncRNA molecule (called *Gomafu*)
32 with a characteristic expression pattern in the mouse nervous system localized in the cell nucleus.
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5. IMPLICATIONS FOR FUTURE WORK

An increasing amount of data indicates that miRNAs act as key regulators in a large number of differential cellular processes (Fiore et al., 2008). Furthermore, a variety of miRNAs has already been found in the CNS, of which the list is still expanding. Due to their crucial role in brain development and plasticity and broad genetic regulatory influence, growing evidence suggests that miRNAs may prove to be an important key to our understanding of the pathophysiology and – ultimately – the therapeutics of PD. Investigating the role of miRNAs in disease biology is a new frontier in biomedical research and further exploration of these ncRNAs in the etiology of PDs will benefit from the implementation of additional high throughput experimental approaches. In this context, there are three key complementary issues to consider for further studies.

First, at present, the systematic analysis of miRNA expression has been carried out in a limited number of types and models of PDs. Comprehensive analyses of miRNA expression profiles in post-mortem brains of PD patients of epidemiological relevance (bipolar disorder and major depression, for example) will accelerate the identification of additional deregulated miRNAs (Fiore et al., 2008), and further exploration of miRNA expression profiles in pharmacological and genetic models of PDs will help to elucidate the transcriptional networks underlying PDs.

Apart from expanding the different PDs under investigation, considering the common-disease/common-variant and the common-disease/rare-variants hypotheses (Bodmer and Bonilla, 2008), further systematic studies of novel rare variations (using DNA sequencing) and common known variations (using DNA genotyping) in miRNA genes and their target gene binding sites is a second key issue that will help to identify additional ncRNA based risk factors for PDs. Recent developments in novel high-throughput methodologies for DNA sequencing and genotyping have created the opportunity to implement such approaches. These studies can be complemented by investigating variations in genes involved in the miRNA processing machinery and of CNVs in miRNA regions. In this context, the recent observations that miRNAs can also regulate the function of target genes through binding to their promoter and coding regions (Place et al., 2008;

1 Tay et al., 2008), have important implications for the design and interpretation of these genetic
2 studies.
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6 As a final issue to fully comprehend the involvement of miRNAs in the etiology of PDs, we wish
7 to emphasize the importance to confirm predicted functional effects of the deregulation of PD-
8 associated miRNAs. This confirmation will greatly depend on the implementation of systematic
9 analyses of behavioural and molecular profiles of other animal models (e.g. mice and flies) with
10 genetic manipulations in candidate brain-expressed miRNAs and other genes involved in miRNA
11 processing. A promising approach there is the targeted knock-down of specific disease related
12 miRNAs using anti-miRNA oligos (miRNA inhibitors) (Zhang, 2008).
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23 Although only a limited number of candidate miRNAs and UTRs have been studied in PD
24 patients previously, these results clearly highlight the importance of exploring additional
25 nonprotein-coding regions. We propose that future functional and genomic explorations of brain
26 expressed miRNAs, and of other types of non-coding RNAs, will facilitate the identification of
27 additional candidate genes and pathways for common PDs.
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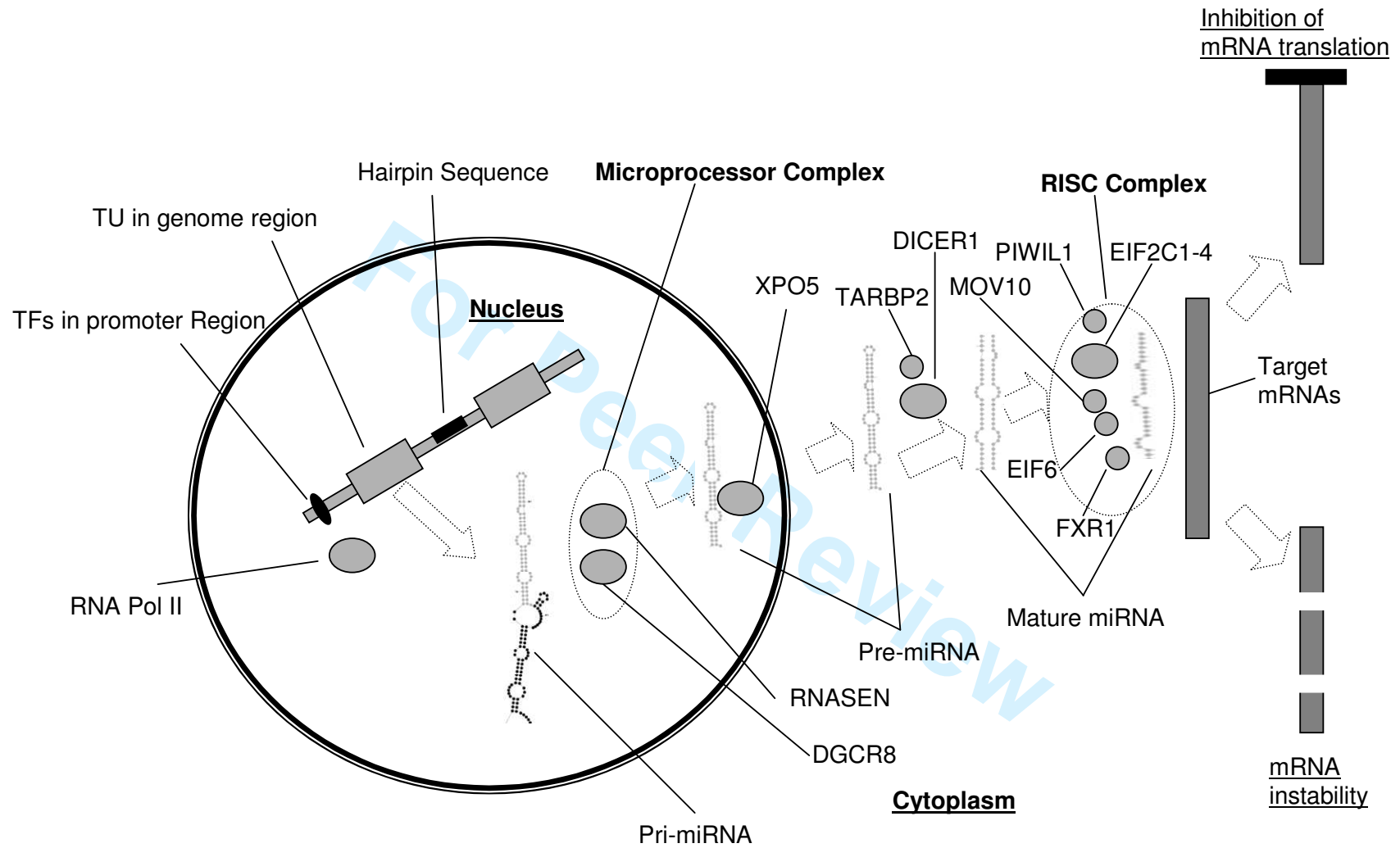
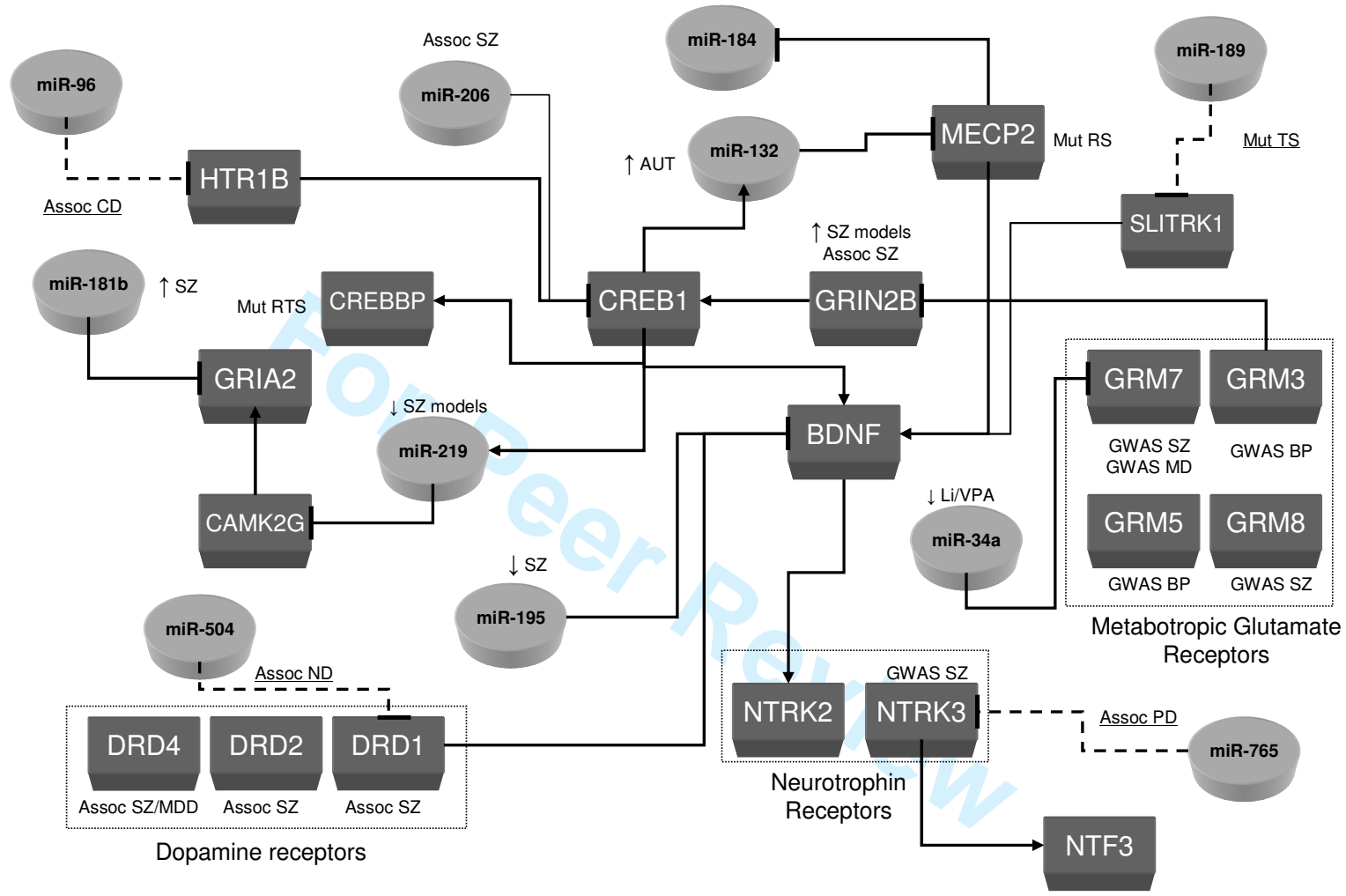


Figure 1. Known molecular mechanisms implicated in miRNA generation and processing. RNA pol II: RNA polymerase II; TU: Transcriptional Unit; TF : Transcription Factor.



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Figure 2: Overview of miRNA-regulated signalling networks of specific importance to brain plasticity and involved in psychiatric disorders. Protein coding genes are indicated in dark grey, miRNAs are shaded light grey. Positive and negative interactions are represented as arrows (→) and blunt ending lines (---), respectively. Dotted lines represent miRNA-interactions with known-allele dependent effects. Their associated phenotypes are underlined.

BDNF: Brain-derived neurotrophic factor; CAMK2G: Calcium/calmodulin-dependent protein kinase II gamma; CREB1: cAMP responsive element binding protein 1; CREBBP: CREB binding protein; DRD1: Dopamine receptor D1; DRD2: Dopamine receptor D2; DRD4: Dopamine receptor D4; GRIA2 : Glutamate receptor, ionotropic, AMPA 2; GRIN2B: Glutamate receptor, ionotropic, N-methyl D-aspartate 2B; GRM3: Glutamate receptor, metabotropic 3; GRM5: Glutamate receptor, metabotropic 5; GRM7: Glutamate receptor, metabotropic 7; GRM8: Glutamate receptor, metabotropic 8; HTR1B: Serotonin receptor 1B; MECP2: Methyl CpG binding protein 2; NTF3: Neurotrophin 3; NTRK2: Neurotrophic tyrosine kinase, receptor, type 2; NTRK3: Neurotrophic tyrosine kinase, receptor, type 3; SLITRK1: Slit and NTRK-like family, member 1. ND: Nicotine dependence; PD: Panic Disorder; Li/VPA: Lithium/Valproate, AUT: Autism; MDD: Major depression, BP: Bipolar Disorder; SZ: Schizophrenia; RTS: Rubenstein-Taybi syndrome; TS: Tourette syndrome; RS: Rett syndrome; CD: Conduct-disorder-related behaviours; Assoc: Association identified in meta-analyses; Mut: Mutated in; ↓: Down-regulated levels; ↑: Up-regulated levels.

microRNAs	Model	Phenotype	Reference
miR-132	Neonatal rat cortical neurons	Over-expression led to enhancement of neurite outgrowth; via p250GAP, miR-132 is regulated by CREB	(Vo et al., 2005; Wayman et al., 2008)
miR-134	Cultured rat hippocampal neurons	Over-expression led to a diminution of dendritic spine volume and width; it regulates Limk1	(Schratt et al., 2006)
miR-124	Neuronal cell lines	Over-expression led to enhancement of neuronal differentiation and incorporation of neural-specific exons in some genes	(Makeyev et al., 2007)
miR-138	Rat hippocampal neurons	Inhibition led to an increase in dendritic spine volume	(Siegel et al., 2009)
miR-125b	Neuronal cell lines	Over-expression led to enhancement of neuronal differentiation	(Le et al., 2009)
miR-125a	P19 cells	Increased levels in neuronal differentiation induced by retinoic acid	(Wu and Belasco, 2005)
miR-124	Neuronal cell lines	Increased levels in neuronal differentiation induced by retinoic acid	(Hohjoh and Fukushima, 2007)
miR-219	Circadian rhythm in mouse	An antagomiR shortened the circadian period length	(Cheng et al., 2007)
miR-30a, miR-195	Rat neurons and human prefrontal cortex	These miRNAs down-regulated BDNF and showed inverse correlations with levels of BDNF protein in human prefrontal cortex	(Mellios et al., 2008b)
let-7b, let-7d	Rats	Up-regulated in hippocampus of sleep deprived rats	(Davis et al., 2007)
miRNA-200 Family	Rats	Specific expression in olfactory neurons	(Choi et al., 2008)
miR-24, miR-92, miR-204, miR-325, miR-293, miR-181-b, miR-214, miR-292-5p, miR-299	Hippocampal slices from mice	Up-regulated by LTP	(Park and Tang, 2009)
miR-92, miR-215, miR-290, miR-297, miR-324-5p, miR-340, miR-200-c, miR-218, miR-466, miR-31	Hippocampal slices from mice	Up-regulated by LTD	(Park and Tang, 2009)
miR-284	Neuromuscular junctions in <i>Drosophila</i>	Knockout of miR-284 led to up-regulation of GluIIRA and GluIIRB (two glutamate receptor subunits)	(Karr et al., 2009)
miR-430	Zebrafish	Reversion of brain alterations in KO models for dicer	(Giraldez et al., 2005)
miR-273	<i>C. elegans</i>	Control of differentiation of taste receptor neurons	(Johnston et al., 2005)

Table 1: Overview of microRNAs known to be related to basic mechanisms of brain and neural function.

microRNA	Organisms	Disorder	Evidence	Reference
miR-181b	Human	Schizophrenia	Up-regulated in post-mortem brains	(Beveridge et al., 2008)
miR-26b/miR-92/miR-24/miR-30e	Human	Schizophrenia	Down-regulated in post-mortem brains	(Perkins et al., 2007)
miR-346	Human	Schizophrenia	Down-regulated in post-mortem brains	(Zhu et al., 2009)
miR-195	Human	Schizophrenia	Down-regulated in post-mortem brains	(Mellios et al., 2008a)
miR-219	Mouse	Schizophrenia	Down-regulated in the prefrontal cortex of mice with pharmacological and genetic inhibitions of NMDA signalling	(Kocerha et al., 2009)
miR-185, miR-134, miR-299, miR-324-5p, miR-491, miR-532	Mice	Schizophrenia	Mature miRNAs down-regulated in a mouse model for schizophrenia (microdeletion of 22q11.2)	(Stark et al., 2008)
miR-199a, miR-128a, and miR-128b	Rats	Schizophrenia	Up-regulated in brains of rats treated with haloperidol	(Perkins et al., 2007)
miR-221, miR-152, miR-15a, miR-155, miR-181c, miR-34a	Human	Bipolar disorder	Up-regulated in human lymphoblastoid cell lines treated with lithium	(Chen et al., 2009)
miR-494	Human	Bipolar disorder	Down-regulated in human lymphoblastoid cell lines treated with lithium	(Chen et al., 2009)
let-7b, let-7c; miR-128a, miR-24a; miR-30c; miR-34a, miR-221	Rats	Bipolar disorder	Down-regulated in brains of rats treated with mood stabilizers (lithium and valproate)	(Zhou et al., 2009)
miR-144	Rats	Bipolar disorder	Up-regulated in brains of rats treated with mood stabilizers (lithium and valproate)	(Zhou et al., 2009)
miR-484	Human	Autism spectrum disorders	Down-regulated in post-mortem brains	(Abu-Elneel et al., 2008)
miR-23a, miR23b, miR-132, miR-146a, miR-146b, miR-663	Humans	Autism spectrum disorders	Up-regulated in human lymphoblastoid cell lines of patients with autism	(Talebizadeh et al., 2008)
miR-92, miR-320, miR-363	Humans	Autism spectrum disorders	Down-regulated in human lymphoblastoid cell lines of patients with autism	(Talebizadeh et al., 2008)
miR-132	Mouse	Rett syndrome	Down-regulated in brains of MECP2 knockout mice. In addition, miR-132 regulates MECP2.	(Klein et al., 2007)
miR-18a	Rats	Mood and anxiety disorders	Up-regulated in the paraventricular nucleus of Fischer 344 rats exposed to repeated restraint stress	(Uchida et al., 2008)
miR-134	Rats	Mood and anxiety disorders	Down-regulated in brains of 344 rats exposed to chronic immobilization stress	(Meerson et al., 2009)
miR-124 and let-7a	Rats	Substance abuse disorders	Down-regulated in brains rats exposed to psychoactive drugs	(Chandrasekar and Dreyer, 2009)

Table 2: Overview of microRNAs reported to be dysregulated in tissues of patients with psychiatric disorders and/or animal and cell models.

miRNA	Location	Variation	Phenotype	Population	Frequency (%) Cases/Controls	Function	Reference
miR-222	Chr Xp11.3	C/T-pri-miR	X-linked Mental Retardation (138)	USA	0.36/-	Not reported	(Zhang et al., 2006)
miR-222	Chr Xp11.3	A/T-C/T-pre-miR	Nonsyndromic X-linked mental retardation	European countries	0.24/0	Not reported	(Chen et al., 2007)
miR-130b	Chr 22q11.21	C/G-pri-miR (rs861843)	Schizophrenia (300 cases, 316 controls)	Russia	14.5/14.4	Predicted changes in TF binding	(Burmistrova et al., 2007)
miR-206	Chr 6p12.2	C/T-pri-miR (rs17578796)	Schizophrenia (840 cases and 1476 controls)	Scandinavian countries	1.6/0.4	Not reported	(Hansen et al., 2007)
miR-502	Chr Xp11.23	C/G-pre-miR	Schizophrenia (193 male patients and 191 male controls)	USA	1 patient/0	Lower mature and pre-miR levels	(Feng et al., 2009)

Table 3: Overview of published genetic variants in human microRNA genes associated with psychiatric disorders.

3'UTR (miR)	Location	Variation	Phenotype	Population	Frequency (%) Cases/Controls	Functional effects of mt allele	Reference
SLITRK1 (miR-189)	Chr 13q31.1	G/A (+689)	Tourette's syndrome (174 cases, 2148 controls)	USA	0.57/0.0	Higher inhibition of gene expression	(Abelson et al., 2005)
HTR1B (miR-96)	Chr 6q14.1	A/G (rs13212041)	Quantitative measures of conduct-disorder-related behaviours (359 subjects)	USA	-/16	Lower inhibition of gene expression	(Jensen et al., 2009)
DRD1 (miR-504)	Chr 5q35.2	A/G (rs686)	Nicotine dependence (2037 individuals)	USA	-/40.5	Lower activation of gene expression	(Huang and Li, 2009)
NTRK3 (miR-485-3p)	Chr 15 q25.3	C/G rs28521337	Obsessive compulsive disorder (153 patients and 324 controls)	Spain	40/47	No differences in inhibition of gene expression	(Muinos-Gimeno et al., 2009)
NTRK3 (miR-128 and miR-509)	Chr 15 q25.3	T/C (ss102661458)	Panic Disorder (212 patients and 324 controls)	Spain	1 patient/0	Lower inhibition of gene expression	(Muinos-Gimeno et al., 2009)
NTRK3 (miR-765)	Chr 15 q25.3	G/C (ss102661460)	Panic Disorder (212 patients and 324 controls)	Spain	1 patient/0	Lower inhibition of gene expression	(Muinos-Gimeno et al., 2009)
HTR1B (miR-96)	Chr 6q14.1	A/G (rs13212041)	Self-reports of anger and hostility in young men (361 subjects)	USA	-/16	Lower inhibition of gene expression	(Conner et al., 2009)

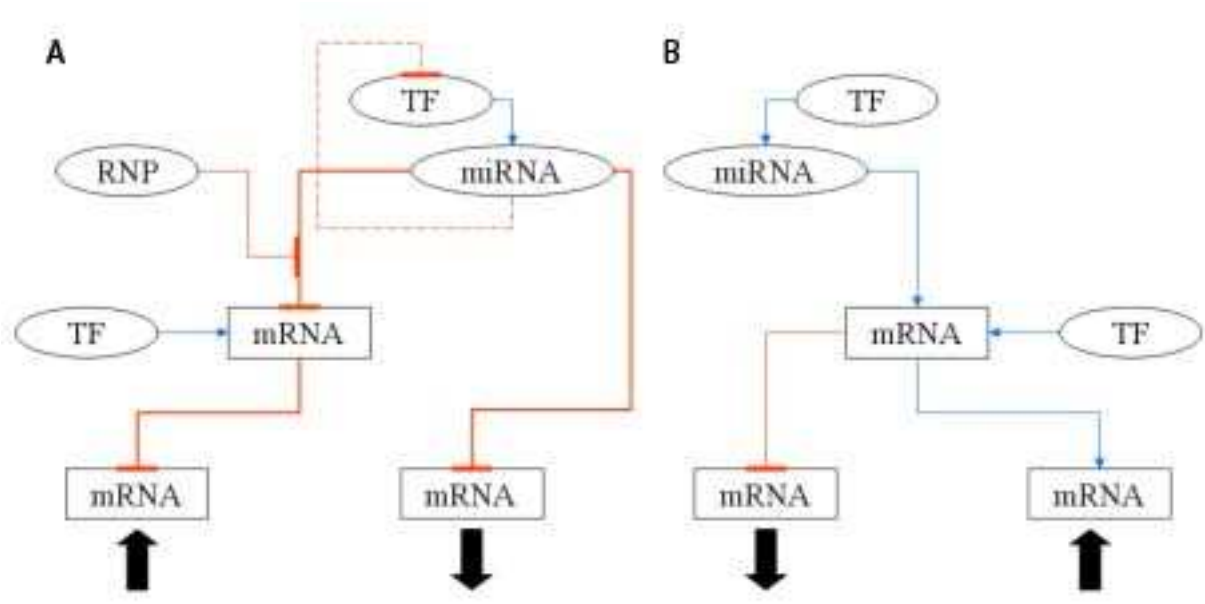
Table 4: Overview of published genetic variants in miRNA binding sites in 3'UTR regions of candidate genes associated with psychiatric disorders in humans.

Gene Symbol	Name	Chromosome	Genomic Length (bp)	Exons	Protein (aa)	SNPs (1)	CNVs (2)	Tissue (3)
DICER1 (Dicer1)	Dicer1	14q32.13	71,193	28	1912	175	Yes	Spinal Cord
RNASEN (Drosha)	Ribonuclease III, nuclear	5p13.3	130,999	35	1374	718	Yes	Fetal Brain
DGCR8 (Pasha)	DiGeorge syndrome critical region gene 8	22q11.21	31,586	14	773	180	Yes	Cerebellum
XPO5 (Exportin-5)	Exportin 5	6p21.1	53,679	32	1204	193	Yes	Fetal Brain
EIF2C1 (hAgo1)	Eukaryotic translation initiation factor 2C, 1	1p35-p34	54,491	19	857	112	No	Cerebellum Peduncles
EIF2C2 (hAgo2)	Eukaryotic translation initiation factor 2C, 2	8q24.3	104,381	19	851	557	No	Dorsal Root Ganglia
EIF2C3 (hAgo3)	Eukaryotic translation initiation factor 2C, 3	1p34.3	125,745	19	860	496	No	Prefrontal Cortex
EIF2C4 (hAgo4)	Eukaryotic translation initiation factor 2C, 4	1p34.3	48,593	18	861	252	No	Prefrontal Cortex
MOV10 (Armitage)	Moloney leukemia virus 10, homolog	1p13.2	26,325	20	1003	97	Yes	Cerebellum
EIF6	Eukaryotic translation initiation factor 6	20q12	6,074	7	245	66	No	Spinal Cord
FXR1	Fragile X mental retardation, autosomal homolog 1	3q28	64,498	16	621	292	No	Hypophysis
TARBP2 (Loqs)	TAR (HIV-1) RNA binding protein 2	12q12-q13	5,510	9	366	23	No	Cerebellum
PIWIL1 (Aubergine)	Piwi-like 1	12q24.33	34,260	21	861	266	Yes	Pons
PIWIL2 (Ago3)	Piwi-like 2	8p21.3	80,774	23	973	471	Yes	Prefrontal Cortex
PIWIL3 (Ago3)	Piwi-like 3	22q11.23	55,683	21	882	707	No	Cingulate Cortex
PIWIL4 (Ago3)	Piwi-like 4	11q21	54,073	20	852	449	No	Cerebellum

Supplementary Table 1: Summary of human miRNA processing machinery subunit genes. Names between brackets correspond to those genes and proteins identified in experimental models. (1) Data obtained from dbSNP (Sherry et al, 2001). (2) Data obtained from the Database of Genomic Variants (Iafate et al, 2004). (3) Neural tissue with the highest expression (taken from Su et al, 2004).

miRNA ID	#	miRNA ID	#	miRNA ID	#
hsa-miR-26a	1	hsa-miR-126	18	hsa-miR-21	35
hsa-miR-125b	2	hsa-miR-23b	19	hsa-miR-221	36
hsa-miR-9	3	hsa-miR-30a-5p	20	hsa-miR-132	37
hsa-miR-16	4	hsa-let-7f	21	hsa-miR-191	38
hsa-miR-100	5	hsa-miR-124a	22	hsa-miR-342	39
hsa-let-7c	6	hsa-let-7a	23	hsa-miR-15a	40
hsa-miR-30c	7	hsa-miR-222	24	hsa-miR-25	41
hsa-miR-181b	8	hsa-miR-181a	25	hsa-miR-103	42
hsa-miR-321	9	hsa-miR-20	26	hsa-let-7b	43
hsa-miR-99a	10	hsa-miR-19b	27	hsa-miR-223	44
hsa-miR-26b	11	hsa-miR-27b	28	hsa-miR-137	45
hsa-miR-125a	12	hsa-miR-181d	29	hsa-miR-19a	46
hsa-let-7g	13	hsa-miR-30b	30	hsa-miR-149	47
hsa-miR-92	14	hsa-miR-204	31	hsa-miR-126*	48
hsa-miR-124b	15	hsa-miR-195	32	hsa-miR-127	49
hsa-miR-24	16	hsa-miR-29c	33	hsa-miR-93	50
hsa-miR-29a	17	hsa-miR-9*	34	hsa-miR-135a	51

Supplementary Table 2: Brain-enriched human miRNAs (adult brain). Brain-specific miRNAs are indicated in bold (Data derived from Liang et al, 2007).



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Supplementary Figure 1: Possible functional interactions of miRNA regulation processes with transcriptional systems controlling gene expression. Blue lines represent positive interactions and red lines represent negative modulations. TF (transcription factors), RNP (ribonucleoprotein). (A) Possible functional consequences of “classical” (inhibitory) direct effects of miRNAs on gene expression. TFs can activate the expression of miRNAs (e.g. CREB and miR-132). These miRNAs can inhibit the expression of the transcription factors in a feedback loop (e.g. miR-133 and PITX3). The inhibitory direct effects of miRNAs on mRNA expression can be modulated by ribonucleoproteins (e.g. ELAVL1 on SLC7A1/miR-122). miRNA-dependent down-regulation of genes that have inhibitory effects on gene expression (e.g. miR-124 and MECP2) can lead to an indirect up-regulation of other genes (e.g. miR-124 and PTBP2). (B) Novel functions of some miRNAs, activating gene expression (e.g. miR-373 and CDH1).

	miR-9	miR-181a	miR-124a	miR-204	miR-132	miR-137	miR-149	miR-125b	miR-100	miR-221	miR-191	miR-342	miR-103	miR-127	miR-135a
D. melanogaster															
M. musculus															
H. sapiens															

Supplementary Figure 2: Phylogenetic conservation patterns of adult brain-enriched and brain-specific (in bold) miRNAs. Blue indicates homologues exists while red indicates that no homologue is present.

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