# Determining the role of microRNAs in psychiatric disorders

*Orna Issler[1,2] and Alon Chen[1,2]*

Abstract | Recent studies have revealed that patients with psychiatric disorders have altered microRNA (miRNA) expression profiles in the circulation and brain. Furthermore, animal studies have shown that manipulating the levels of particular miRNAs in the brain can alter behaviour. Here, we review recent studies in humans, animal models, cellular systems and bioinformatics that have advanced our understanding of the contribution of brain miRNAs to the regulation of behaviour in the context of psychiatric conditions. These studies highlight the potential of miRNA levels to be used in the diagnosis of psychiatric disorders and suggest that brain miRNAs could become novel treatment targets for psychiatric disorders.

Epigenetic mechanisms
Molecular mechanisms leading to changes in gene expression levels that are not due to variations in the DNA sequence.

[1]*The Ruhman Family Laboratory for Research on the Neurobiology of Stress, Department of Neurobiology, Weizmann Institute of Science, 76100 Rehovot, Israel.*
[2]*Department of Stress Neurobiology and Neurogenetics, Max Planck Institute of Psychiatry, 80804 Munich, Germany.*
*Correspondence to A.C.*
*e-mails: alon.chen@weizmann.ac.il; alon_chen@psych.mpg.de*
doi:10.1038/nrn3879

Most psychiatric disorders display a strong genetic component, but heritability by itself can only partially explain an individual's risk of developing a mental disorder[1–3]. Only a few specific gene mutations have been directly linked to increased susceptibility for such conditions[4,5]. Moreover, epidemiological studies have reported associations between environmental factors — mainly exposure to psychological or physiological stressors — and psychiatric morbidity[6–9]. For example, stress *in utero* or during early life may programme the brain to become more vulnerable to particular psychiatric disorders, whereas stress in adolescence or later in adulthood may trigger the onset of such disorders[10–12]. Thus, a complex interaction between genetic predisposition and environmental factors is suggested to be at the root of mental disorders.

Environmental factors can, through epigenetic mechanisms, induce changes in gene expression levels that might mediate the onset of a disease without altering the DNA sequence[13,14] (FIG. 1). These mechanisms include histone modification, DNA methylation and post-transcriptional regulation by non-coding RNAs such as microRNAs (miRNAs), which are the focus of this Review. Elucidating the role of epigenetic processes in mediating CNS functions may promote a better understanding of the pathophysiology and neurobiology of psychiatric disorders and could thereby promote much-needed breakthroughs in the development of new drug targets and biomarkers for these illnesses.

Since the discovery of miRNAs, descriptions of novel miRNAs and their widespread role in biological processes have accumulated, and this has revealed miRNAs to be a prevalent mode of post-transcriptional regulation of gene expression[15]. Each miRNA can regulate up to hundreds of downstream targets. Collectively, miRNAs are predicted to regulate more than half of all protein-coding genes and to affect many cellular processes in health and disease[16]. Much is already known about miRNA biogenesis[17] and function[15,18,19] (BOX 1); briefly, a mature single-stranded miRNA, of about 22 nucleotides, is incorporated into a silencing complex, and this induces translational repression or destabilization of the target mRNA. miRNAs can act as an 'expression switch' and block the expression of their target genes[15]. In such cases, a mutually exclusive expression pattern of the silencing miRNA and its target genes is often observed, as is commonly reported in developmental studies. Alternatively, miRNAs can act as 'fine-tuners' of the expression levels of their target genes, as observed when they are co-expressed along with their target genes — a phenomenon that is often reported in adult tissues[15,20]. Indeed, adult brain miRNAs function as endogenous 'hubs' for the fine-tuning of target gene expression and thereby affect the structure and function of neuronal networks.

Neuroscience research has primarily focused on miRNAs in the context of development (reviewed in REF. 21), neurodegenerative disorders (reviewed in REFS 22–24) and synaptic plasticity (reviewed in REFS 25–27). In this Review, we focus on the role of miRNAs in the regulation of behaviour, emotion and cognition, specifically in psychiatric disorders, in which this regulation is disturbed. This is the first era of the study of miRNA biology in psychiatry. Data are rapidly accumulating regarding miRNAs as mediators of the onset, susceptibility, diagnosis and treatment of mental disorders.

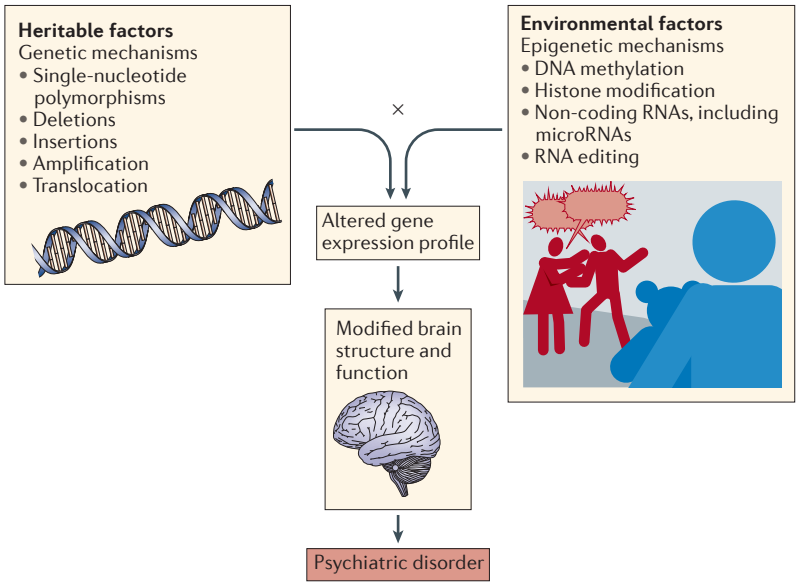



Figure 1 | **A working hypothesis of the aetiology of psychiatric disorders.** A combination of genetic factors (including polymorphisms, gene deletions or insertions, gene amplification and gene translocation) and environmental factors (mainly stress) may lead to a predisposition for the development of psychiatric disorders. Epigenetic mechanisms mediate the effect of the environment on gene expression. Altered gene expression caused by genetic and epigenetic mechanisms modifies brain structure and function and, in doing so, facilitates the occurrence of psychiatric disorders. MicroRNAs are one of several mechanisms through which environmental factors may influence gene expression and subsequent behaviour and physiology.

## Experimental approaches

Studies on the role of miRNAs in psychiatric disorders have collected data from human patients, animal models of these disorders and cellular systems, and have used various approaches. Some approaches are unique to the study of miRNAs, whereas others are modifications of research techniques that are used to study protein-coding genes[28]. Some procedures are common to human, rodent and cellular studies, whereas others are unique to one type of model.

Expression profiles of miRNAs are often analysed using high-throughput methods (reviewed in REF. 29). Bioinformatics analysis is used to identify specific miRNA–target gene interactions. *In vitro* assays are often carried out to confirm the predicted interactions and to provide an in-depth understanding of miRNA-related molecular mechanisms. Furthermore, mouse models are used to test causal effects of alteration of specific miRNA expression levels on mouse behaviour, physiology and gene expression.

By and large, studies combining several approaches and using different models provide the greatest validity. So far, only a few comprehensive studies of miRNAs in psychiatric disorders have been carried out; most miRNA studies in this context have been limited to a single level of analysis. Below, we discuss the pros and cons of the common approaches that have been used in these investigations (TABLE 1). We also highlight specific studies that used broader approaches to gain insights into the role of a specific miRNA in psychiatric disorders (TABLE 2).

Copy number variations (CNVs). Genetic variations in which large areas in the genome are deleted or duplicated.

Single-nucleotide polymorphisms (SNPs). Common genetic variations occurring in one base pair.

Seed match sequences Short sequences within the 3′untranslated region of a target gene (seed match sequence) that are complementary to sequences within a microRNA (the seed sequence); the seed and seed match sequences direct the specificity of the miRNA–target gene interactions.

Genome-wide association study (GWAS). A study that correlates common genetic variations in the entire human genome with a specific disease.

## Human studies

***Genetic association studies.*** Several studies have reported chromosomal deletions, insertions, duplications or copy number variations (CNVs) in regions encoding miRNA biogenesis-related proteins or specific miRNAs that are associated with susceptibility to psychiatric disorders. Furthermore, single-nucleotide polymorphisms (SNPs) in miRNA biogenesis-related genes, in specific miRNA-encoding genomic loci or in seed match sequences of target-gene 3′ untranslated regions (UTRs) have been associated with an increased risk for psychiatric disorders[30,31]. Such variations in miRNA biogenesis-related genes can alter the expression levels of a large subset of miRNAs, whereas variation in specific miRNA-encoding genes can alter their expression level or affect the strength of miRNA–target interactions[32] (FIG. 2). Data from such genetic association studies might have important diagnostic implications; however, the findings are usually of a correlative nature and therefore lack causal evidence. Detailed validation experiments should be carried out, confirming whether the identified variations affect miRNA expression levels or miRNA–target interactions.

A representative example of a SNP in an miRNA gene that has been linked to a psychiatric disorder is the rs1625579 SNP in miR-137. A comprehensive genome-wide association study (GWAS) involving 40,000 individuals showed that rs1625579, which is found within the putative primary miR-137 (pri-miR-137) transcript (BOX 1), is associated with an increased risk of schizophrenia[33]. Notably, the risk allele (T) is far more commonly found in the general population (with about 80% frequency) than the alternative, minor allele (G)[34]. A small post-mortem study showed that healthy controls that were homozygous for the risk allele had lower levels of mature miR-137 in the prefrontal cortex than did individuals with other genotypes[35], suggesting that this SNP affects miR-137 expression levels.

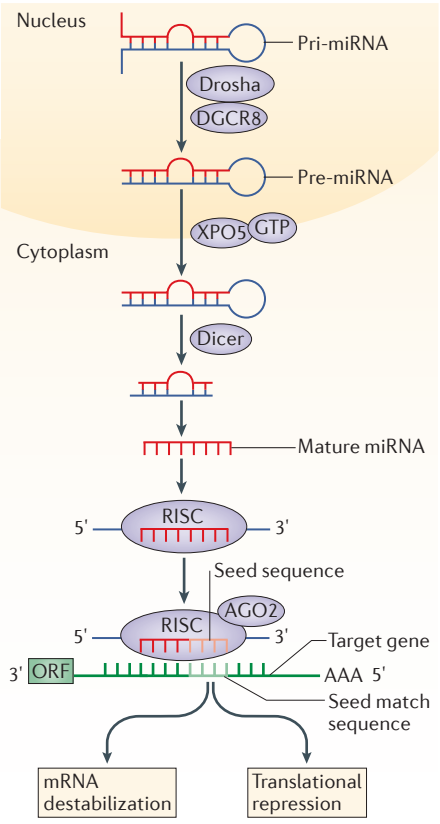
Interestingly, miR-137 had been suggested to have a role in neurogenesis[36] and neuronal maturation[37] before it was linked to schizophrenia. Since this schizophrenia GWAS, several follow-up studies involving large numbers of individuals have been conducted (reviewed in REF. 38). The results were replicated[39] and suggest that the risk-allele carriers who have schizophrenia are a subgroup of patients with fewer positive and more negative symptoms of the disorder than patients who do not carry the risk allele[40,41]. In addition, individuals with schizophrenia who are homozygous for the risk allele have an earlier onset of illness and a more pronounced disruption in brain structure (reduced white-matter integrity throughout the brain, smaller hippocampi and larger lateral ventricles)[42]. Furthermore, functional MRI (fMRI) studies have shown that the pri-miR-137 genotype influences the activity of the posterior right medial frontal gyrus during a cognitive task[43] and affects fronto-amygdala and dorsolateral prefrontal–hippocampal functional connectivity during emotional tasks[44] and in the resting state[45], respectively.

Other studies have identified putative miR-137 target genes that might play a part in schizophrenia by overexpressing miR-137 *in vitro* and profiling the resulting

Box 1 | **MicroRNA biogenesis, mode of activity and nomenclature**

In most cases, microRNA (miRNA) genes are transcribed by RNA polymerase II and RNA polymerase III into primary miRNA transcripts (pri-miRNA)[107]. Pri-miRNAs are either transcribed from dedicated genes or processed from introns of other genes as individual miRNAs or miRNA clusters, and they form distinctive secondary hairpin structures (see the figure). This pri-miRNA is then cleaved by a microprocessor complex that contains Drosha (also known as ribonuclease 3) and the microprocessor complex subunit DiGeorge syndrome critical region 8 (DGCR8)[108] (see the figure). Drosha cleaves the 5′ and 3′ arms of the pri-miRNA hairpin, whereas DGCR8 directly interacts with and stabilizes the pri-miRNA and serves as a molecular ruler to determine the precise cleavage site. Following cleavage of the RNA molecule, its size is reduced to 70–110 nucleotides in length and it is referred to as precursor miRNA (pre-miRNA). After nuclear processing, the pre-miRNA is exported to the cytoplasm by exportin 5 (XPO5) in a complex with RAS-related nuclear protein (RAN)–GTP[109]. The pre-miRNA is then cleaved by Dicer to generate a ~22-nucleotide miRNA duplex. Following Dicer-mediated cleavage, Dicer and its interaction-domain protein TAR RNA binding protein (TRBP; also known as TARBP2) dissociate from the miRNA duplex to form the active RNA-induced silencing complex (RISC) that performs gene silencing. The double-stranded duplex needs to be separated into the functional guide strand (which is complementary to the target mRNA) and the passenger strand (which is subsequently degraded). The functional strand of the mature miRNA is loaded into the RISC complex together with Argonaute 2 (AGO2)[110]. Only one strand is usually incorporated into the RISC, and it guides the complex to target the 3′ untranslated region (3′ UTR) of the mRNAs to inhibit their translation or promote their degradation. The specificity of miRNAs to their target mRNAs is canonically determined by base-pairing of a 6–8-nucleotide sequence in the 5′ end of the mature miRNA (known as the seed sequence) to a complementary seed match sequence in the 3′ UTR end of the target gene[15,69].

Thousands of miRNAs across different species have been identified, and a nomenclature system has therefore been adopted[111]. Briefly, the numbering of newly identified miRNA genes is sequential and is attached to the prefix 'mir' followed by a dash (for example, mir-135). The uncapitalized 'mir' refers to the pre-miRNA and the capitalized 'miR' refers to the mature form. miRNAs with closely related mature sequences are annotated with lower case letters to show their similar structure (for example, miR-135a and miR-135b). Distinct precursor sequences and genomic loci that express identical mature sequences are indicated with an additional number (for example, miR-135a1 and miR-135a2). miRNAs should also be preceded by the annotation for the species they are observed in (for example, hsa-miR-135 in *Homo sapiens*). miRNAs originating from the 3′ end or 5′ end are denoted with a '-3p' or '-5p' suffix, respectively (for example, miR-135-3p and miR-135-5p). For more information on the nomenclature of miRNA passenger and guide strands, see the miRBase nomenclature guide. ORF, open reading frame.




mRNA expression pattern[46,47]. Interestingly, four of the hits that were identified in the GWAS that linked the pri-miR-137 SNP to schizophrenia are predicted to be targets of miR-137. Indeed, *in vitro* luciferase assays confirmed that miR-137 interacts with *CSMD1* (CUB and Sushi multiple domains 1), *WBP1L* (WW domain binding protein 1-like; also known as *C10ORF26*), *CACNA1C* (calcium channel, voltage-dependent, L type, α1C subunit) and *TCF4* (transcription factor 4)[48]. In addition, two further schizophrenia-related targets of miR-137 were identified and validated *in vitro*: *ZNF804A* (zinc-finger protein 804A)[49] and *RORA* (RAR-related orphan receptor A)[50].

More post-mortem and *in vitro* studies are needed to confirm that the rs1625579 SNP directly affects the expression level of miR-137. In addition, assessing the effects of this SNP *in vivo* in mice carrying the different genotypes, or by experimentally modifying miR-137 expression levels, can shed light on the role of this SNP and miR-137 target genes in schizophrenia-like behaviours.

***Post-mortem studies.*** The miRNA expression profiles in specific brain sites — obtained post-mortem from patients with psychiatric disorders — can be characterized using high-throughput techniques. Such screens are possible owing to the long half-life and relatively low sensitivity to degradation of miRNAs compared with longer RNA molecules[51]. This approach reveals that several psychiatric disorders have characteristic miRNA expression profiles, which suggests that altered miRNA levels might contribute to the pathophysiology of these disorders. The strongest caveat of this approach is its correlative nature; that is, the altered miRNA profile observed in the patient's brain tissue at death does not necessarily reflect a causal role for a particular miRNA in the disease. Indeed, the profile might reflect secondary effects of other alterations associated with a specific pathology or medical treatment. It should be noted that high-throughput platforms for miRNA profiling are not only expensive but also error prone; therefore, their results require additional

Table 1 | **Comparing available approaches for studying the role of miRNAs in psychiatric disorders**

| Approach | Experimental methods | Strengths | Weaknesses |
|---|---|---|---|
| High-throughput screens of miRNA expression profiles | • miRNA microarrays<br>• RNA sequencing<br>• Real-time PCR | Comprehensive | Expensive and error prone |
| Testing regulation of the expression of a specific miRNA | • Real-time PCR<br>• *In situ* hybridization | Sensitive and inexpensive | Low throughput |
| Bioinformatic predictions of miRNA–target interactions | Various web-based algorithms | Cheap | Error prone |
| *In vitro* miRNA–target interaction assay | Luciferase reporter assay | Cheap and causal design | Artificial |
| Association between DNA polymorphism and psychiatric conditions | • DNA sequencing<br>• Real-time PCR | Inexpensive | Correlative |
| Mouse models expressing altered levels of miRNA | • Mutating miRNA biogenesis genes<br>• Transgenic mice overexpressing or underexpressing specific miRNAs<br>• Virus-mediated site-specific manipulation of miRNA levels<br>• Pharmacological administration of miRNA mimics or miRNA inhibitors | Causal design | Time consuming and expensive |

miRNA, microRNA.

experimental validation[29]. Furthermore, subsequent bioinformatics analysis is required to identify potential target genes that might be regulated by the miRNAs found in the screen.

Some recent examples of comprehensive studies using this approach have involved post-mortem tissue analysis of patients with major depression disorder (MDD). One study revealed that, compared with controls, patients with MDD showed downregulation of miR-1202 in prefrontal cortex tissue[52]. This finding was replicated in two independent cohorts, which in addition showed that antidepressant treatment and co-morbidity with anxiety disorders attenuated the downregulation of this miRNA in the MDD group[52]. miR-1202 is primate specific and is enriched in the brain[52], which supports its possible role in mediating higher brain functions that may be affected in MDD. Bioinformatics analysis followed by *in vitro* studies suggested that *GRM4* (glutamate receptor, metabotropic 4) is a relevant target gene of miR-1202 (REF. 52). Another study reported downregulation of miR-135 levels in the serotonergic raphe nucleus of individuals with MDD who had committed suicide[53]. This study aimed to elucidate the role of miR-135 as a regulator of serotonin (5-hydroxytryptamine)-related anxiety and depression phenotypes and showed — using mouse models and *in vitro* experiments — that miR-135 targets both the transcript encoding the serotonin transporter (SERT; also known as the sodium-dependent serotonin transporter and SLC6A4) and the transcript encoding 5-hydroxytryptamine receptor 1A (HTR1A; also known as 5HT1A)[53]. The use of post-mortem tissue to identify miRNAs with a possible role in psychiatric disorders can be a good starting point for a top-down approach study (as exemplified in REF. 52) or as a means of validating findings from rodent models (a bottom-up approach, as reported in REF. 53). Either way, such data are of great importance, especially given the substantial limitation of current animal models for psychiatric disorders, which present weak validity and poor predictive power for drug efficacy in human disease[54].

***Studies of circulating miRNAs.*** miRNAs can be detected in blood cells, plasma and serum (particularly in membrane vesicles, exosomes or bound to proteins). The mechanism by which miRNAs enter the circulation is only partially understood. Evidence suggests that miRNAs may be released by passive leakage from cells that undergo apoptosis or necrosis, or that they may be actively secreted from living cells through lipid structures (such as exosomes, shedding vesicles or apoptotic bodies) or protein–miRNA complexes (containing, for example, high-density lipoproteins, Argonaute 2 (AGO2) or nucleophosmin 1)[55–57] (reviewed in REFS 58,59).

The detection of miRNAs in body fluids may not only provide insights into the pathological and physiological state of the originating cells but also suggest an intriguing hormone-like role for these molecules as secreted gene regulator factors. Evidence is accumulating for a role of secreted miRNAs in intercellular crosstalk and for horizontal transfer of miRNAs[59,60]. The possibility that circulating miRNAs might influence the function of adjacent neuronal populations or other neuronal populations at a distance is exciting. Nevertheless, further investigation is required to determine the cellular mechanisms responsible for the packaging of specific miRNAs into secreted vesicles or with RNA-binding proteins, the cellular specificity in miRNA secretion and uptake, the trigger for miRNA release from neurons and the way that miRNAs are functionally incorporated into target cells.

Certain miRNAs in the blood have been proposed as potential biomarkers to aid the diagnosis of various disorders and to evaluate treatment response. To date, most studies of miRNAs in the blood have primarily reported

correlations between circulating miRNA levels and disease states such as different types of cancers[61] and diabetes[62], but there is increasing evidence of altered patterns of circulating miRNAs being associated with psychiatric disorders (reviewed in REF. 63).

Notably, the use of miRNAs as potential biomarkers has been investigated in MDD. Owing to the high prevalence of the disorder, the delay before antidepressant treatments become effective, the low drug-response rates and the risk of suicide associated with MDD, there is an immense need for effective biomarkers for diagnosis and antidepressant treatment efficacy in MDD. Potential biomarkers include miR-1202 (described above), which was found to be downregulated in post-mortem brain tissue from individuals with MDD[52]. The same study further reported that levels of miR-1202 in the blood could be used to distinguish between selective serotonin reuptake inhibitor (SSRI) responders and non-responders; specifically, miR-1202 levels are initially lower in responders than in non-responders and are upregulated on drug administration in the former but not in the latter[52]. This suggests that miR-1202 levels in the circulation could be used to assess the appropriate antidepressant treatment for a given patient. A recent study showed that levels of miR-135a were also lower in the blood of individuals with depression and were increased after treatment in another cohort of patients with this disorder[53].

Replication studies using bigger cohorts are needed to verify the validity of these findings and to identify additional relevant miRNAs. Taken together, MDD diagnosis and response to antidepressant drugs may be reflected in the 'fingerprint' of circulating miRNAs, which could potentially be used in the clinic for diagnosis and treatment assignment. In addition, these findings, along with earlier data on miR-16 (REF. 64) and many correlative studies (reviewed in REFS 31,65,66), suggest that the mode of action of antidepressants is mediated, at least partially, by miRNAs. However, it should be noted that the normal individual variance of these levels in the healthy population needs to be determined before circulating miRNA levels in patients can serve as a useful biomarker.

Alternatively, patient-derived stable cell lines — for example, lymphoblast lines generated from blood lymphocytes — can be generated from blood samples collected from patients with psychiatric disorders. These cells represent the 'genetic landscape' of the patient's disorder, and studying their miRNA expression profile before and in response to treatment can indicate potential miRNAs that are relevant to the disorder. Furthermore, the miRNA expression profiles of patient-derived cell lines can potentially serve as tools for screening responses to drugs as part of a personalized medicine approach. An important limitation of this approach is that generating a cell line may itself change the cells' epigenetic landscape in a way that is not necessarily relevant to the disorder. Therefore, until this caveat is further investigated, alterations in miRNA expression profiles detected in cell lines from patients cannot be considered to be a primary factor that contributes to the pathophysiology of a particular psychiatric disorder.

## Rodent models

***Profiling miRNA levels.*** Animal models provide an opportunity to take a 'snapshot' of miRNA expression patterns in specific brain sites and correlate them with various behaviours. Alterations in miRNA expression profiles that have been identified in rodent models of psychiatric disorders could suggest specific miRNAs that are involved in the development of these conditions or that could be targeted for the treatment of these disorders. Some of the studies that have identified such changes have used genetic mouse models of

Table 2 | **Examples of miRNA-mediated effects that may be relevant to psychiatric disorders**

| miRNA | Suggested effects | Relevant target genes | Associated brain area | Refs |
|---|---|---|---|---|
| miR-1202 | Predicts response to antidepressants | *GRM4* | PFC | 52 |
| miR-128 | Promotes fear extinction | *Arpp21* | PFC | 95 |
| miR-134 | Inhibits fear learning | *Creb* and *Bdnf* | Hippocampus | 119 |
| miR-135 | Mediates antidepressant action | *Sert* and *Htr1a* | Raphe nucleus | 53 |
| miR-16 | Mediates antidepressant action | *Sert* | Raphe nucleus and locus coeruleus | 64 |
| miR-182 | Inhibits fear learning | *Rac1* and *Cttn* | Lateral amygdala | 120 |
| miR-212 | Inhibits drug seeking | *Mecp2* | Dorsal striatum | 97,121 |
| miR-219 | Promotes schizophrenia-like behaviours | *Camk2g* | PFC | 122 |
| miR-34 | Inhibits stress-induced anxiety | *Crfr1* | Central amygdala | 81 |
| miR-124, let-7d and miR-181a | Modulate cocaine-conditioned place preference | *Bdnf* and *Drd3* | Nucleus accumbens | 123,124 |

*Arpp21*, cyclic AMP-regulated phosphoprotein, 21 kDa; *Bdnf*, brain-derived neurotrophic factor; *Camk2g*, calcium/calmodulin-dependent protein kinase IIg; *Creb*, cyclic AMP-responsive element-binding protein; *Crfr1*, corticotropin-releasing factor receptor 1; *Cttn*, cortactin; *Drd3*, dopamine receptor D3; *GRM4*, glutamate receptor, metabotropic 4; *Htr1a*, 5-hydroxytryptamine receptor 1A; *Mecp2*, methyl CpG-binding protein 2; miRNA, microRNA; PFC, prefrontal cortex; *Rac1*, Ras-related C3 botulinum toxin substrate 1; *Sert*, serotonin transporter.

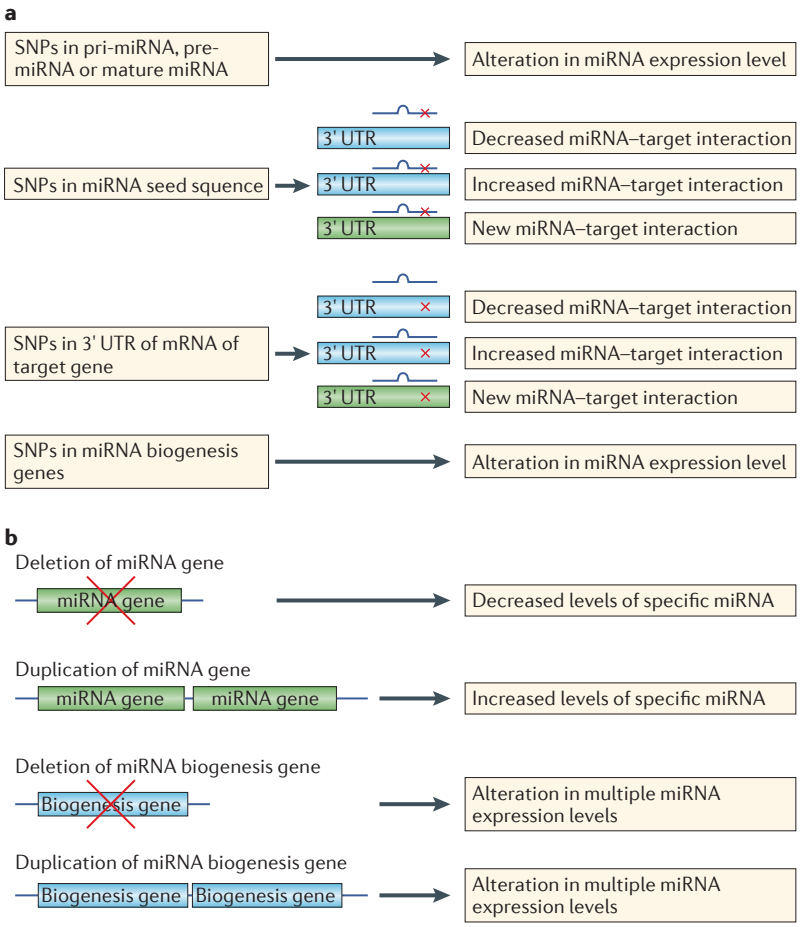



Figure 2 | **MicroRNA-related genetic variations that may be associated with psychiatric disorders. a** | Single-nucleotide polymorphisms (SNPs) in primary microRNA (pri-miRNAs) transcripts, precursor miRNAs (pre-miRNAs), mature miRNAs or other regulatory genomic sites, such as transcription factor binding sites, can alter the expression levels of particular miRNAs. Alternatively, SNPs within the mature miRNA seed sequence or 3′ untranslated region (UTR) of the target gene can strengthen or weaken the interaction between an miRNA and its target, and thereby lead to decreased or increased levels, respectively, of the protein encoded by the target gene. Furthermore, such variation can create new miRNA-related regulatory interplays by creating new seed match sequences that may lead to new miRNA binding sites. Finally, SNPs in the coding region or in regulatory sequences of miRNA biosynthesis genes can alter the expression level of miRNAs and thereby shift the expression of the entire miRNA population[32]. **b** | Chromosomal modification can lead to deletion or duplication of miRNA-encoding genes and thus decrease or increase, respectively, the levels of the miRNA. This will modulate the expression levels of the target genes of such miRNAs accordingly. Similarly, deletion or duplication of genes that are involved in miRNA biogenesis can modify the entire population of miRNAs that rely on that specific gene for their production.

a disorder, whereas others have used environmental manipulations, such as different paradigms of stress, behavioural tasks or different pharmacological treatments that induce particular behaviours that are associated with a psychiatric disorder. Although these approaches are appealing, any changes that are found in the levels of specific miRNAs in such models are correlative and may not reflect a primary causal role for the miRNA in the pathology of interest. In addition, such miRNA profiling does not link specific target genes to the miRNAs modified: confirming any genetic links would require further bioinformatics and experimental work. Isolating miRNAs bound to mRNAs by AGO2 immunoprecipitation and subsequent profiling of both types of transcripts can provide more relevant information regarding the active miRNA–target interactions in the examined tissue[67–69].

The in-depth study of specific miRNA–target interactions at specific brain sites, in specific cell types or in specific circuits has proved to be informative, but a lot more needs to be done to assess the functions of sets of different miRNAs; such sets are routinely reported in miRNA profiling following genetic or environmental manipulations. An example of the use of miRNA profile screens in a genetic model of a neurodevelopmental disorder comes from a study of methyl CpG-binding protein 2 (*Mecp2*)-knockout mice[70]. Mutations in *MECP2* are the main cause of Rett syndrome and are associated with autism[71]. The expression profiles of miRNAs in the cerebellum of *Mecp2*-knockout mice revealed downregulation of a subset of miRNAs, along with altered chromatin methylation of the promoter regions of some of these miRNAs[70]. Furthermore, some of these miRNAs targeted brain-derived neurotrophic factor (*Bdnf*) mRNA *in vitro*, which has been associated with Rett syndrome as well[70]. These findings are in accordance with data from an earlier study that reported that miR-132 targets *Mecp2 in vitro* and is downregulated in the cortex of *Mecp2*-knockout mice[72]. Converging evidence thus suggests a role for a regulatory loop between BDNF, miR-132 and MeCP2 in Rett syndrome and perhaps also in autism spectrum disorders.

Expression profiles can also be used to assess the effects of environmental factors on miRNA expression. For example, recent studies on transgenerational effects of stress in mice showed that offspring of a stressed parent have altered behaviour and epigenetic modifications in the brain, even though they were not directly exposed to stress (reviewed in REF. 73). A study showed that paternal exposure to chronic variable stress in mice altered the activity of the hypothalamic–pituitary–adrenal axis, as well as mRNA and miRNA expression in the paraventricular nucleus of the hypothalamus and the bed nucleus of the stria terminalis in the adult offspring[74]. The sperm of the stressed father also showed changes in miRNA expression, and the authors suggested that this potentially mediated the altered phenotype in their offspring[74]. Similarly, another study showed that exposure to maternal separation increased anxiety and depression-like behaviour, altered blood glucose and insulin levels and modified sperm, hippocampal and hypothalamic RNA levels — including miRNA profiles — in male mice[75]. Furthermore, injecting sperm miRNA from a stressed male into oocytes fertilized by non-stressed parents caused the stress-induced phenotype to appear in the offspring, which showed altered metabolism and behaviour that were similar to those observed in the stressed father[75]. These findings demonstrate the inheritance of acquired traits through miRNAs, thus providing a possible molecular mechanism for transgenerational behavioural plasticity.

AGO2 immunoprecipitation
A method for co-isolation of mRNA and microRNA (miRNA) from cells or tissues that are associated with the Argonaute 2 (AGO2) protein; it allows 'capturing' of active miRNA–target (mRNA) interactions under specific experimental conditions.

***Manipulating the miRNA biogenesis machinery.*** In the early days of miRNA research, a common approach was to test the effects on mouse phenotypes of manipulating key enzymes from the miRNA biogenesis pathway (BOX 1). For example, knocking out *Dicer1* or *Ago2* in a specific brain area or cell type — thereby ablating most of the cellular miRNA functions — is useful as a proof of concept for revealing the involvement of miRNAs in specific biological processes. However, using this approach in the nervous system led to neuronal cell death in most of these studies, especially when the relevant genetic deletion was induced during embryonic development[76–80]. This suggests that abolishing the entire miRNA population is too broad an approach for the investigation of the role of miRNAs in neurons. Nevertheless, there are reports on neuronal survival following *Dicer* depletion in the adult brain[81–83]. Currently, milder and more specific approaches are used, such as manipulating the expression levels of specific miRNAs (see below).

A substantial body of data has accumulated linking the genetic deletion of the miRNA biogenesis gene DiGeorge syndrome critical region gene 8 (*DGCR8*) (BOX 1) with schizophrenia. Microdeletion of the human 22q11.2 locus, which contains *DGCR8,* leads to a genetic syndrome that is associated with a high risk of developing emotional difficulties and schizophrenia[84]. A subsequent study of a mouse model with an equivalent DNA microdeletion (*Df(16)A*$^{+/-}$ mice) suggested that impaired miRNA processing might have a role in this syndrome[85]. The *Df(16)A*$^{+/-}$ mice had reduced levels of mature miRNAs in the brain and showed schizophrenia-associated behaviours such as hyperactivity, decreased sensorimotor gating and working memory deficits[85]. One of the genes lost in this microdeletion is *Dgcr8*; hence, the authors hypothesized that *Dgcr8* haploinsufficiency caused the phenotype.

The downregulation of miRNAs found in *Df(16)A*$^{+/-}$ mice is in line with a study reporting reduced miRNA levels in the prefrontal cortex of patients with schizophrenia post-mortem[86]. However, two other independent studies reported evidence of increased miRNA biogenesis in post-mortem brains from patients with schizophrenia compared with control brains: one study[87] found increased miRNA and *Dicer* mRNA expression in the dorsolateral prefrontal cortex, and the other[88] found increased levels of miRNAs and *Dgcr8* mRNA expression in the superior temporal gyrus and the dorsolateral prefrontal cortex. These conflicting results might be explained by the fact that cases of schizophrenia that are associated with the microdeletion of 22q11.2 account for only 1–2% of the patient population[84,89].

Mice with *Dcgr8* haploinsufficiency (*Dcgr8*$^{+/-}$ mice) showed a reduction in miRNA expression levels, have a behavioural phenotype similar to that of *Df(16)A*$^{+/-}$ mice, and exhibit morphological and functional changes in neurons[85]. There are reports that *Dcgr8*$^{+/-}$ mice have fewer spines and lower dendritic complexity[85], increased long-term potentiation[90] and reduced adult neurogenesis in the hippocampus[91]. Furthermore, the *Dcgr8*$^{+/-}$ mice have fewer neurons, and these neurons have small spines[92], reduced dendritic complexity and altered electrical properties of pyramidal frontal neurons[93] in the cortex. Furthermore, *Dcgr8* haploinsufficiency is likely to affect other 22q11.2 genes, such as the gene encoding miR-185, to produce the deficits associated with 22q11.2 microdeletion. Accordingly, miR-185 is downregulated in *Df(16)A*$^{+/-}$ mice, and overexpression of miR-185 in *Df(16)A*$^{+/-}$ mice rescues their schizophrenia-related aberrant neuronal morphology phenotype[94].

***Manipulating specific miRNA expression levels.*** Several approaches are available for inducing constitutive or transient changes in miRNA levels with different degrees of spatiotemporal resolution (FIG. 3). Germline transgenic approaches include generating loss-of-function mutations in specific miRNA genes in mice (that is, knockout mice) and generating gain-of-function mutations in mice (that is, miRNA-overexpressing mice) in a constitutive, conditional or inducible manner. These models might be associated with developmental compensatory changes but benefit from high reproducibility. Alternative approaches involve stereotaxic injections of either recombinant viruses or modified nucleic acids that induce constitutive or transient effects on miRNA function, respectively. These approaches provide high spatial resolution but suffer from low reproducibility.

Site-specific infusion of an miRNA and an anti-miRNA was used in a study[64] that investigated the mechanisms through which miR-16 targets the transcript encoding SERT and mediates the activity of SSRIs. miR-16 was demonstrated to be expressed at higher levels in noradrenergic cells than in serotonergic cells, and reduced levels of miR-16 in noradrenergic neurons caused *de novo* SERT expression[64]. Chronic treatment with an SSRI in mice increased miR-16 levels in serotonergic raphe nuclei, which reduced SERT expression. Interestingly, raphe exposed to SSRIs released the neurotrophic factor S100β, which acts on noradrenergic cells of the locus coeruleus. Decreasing levels of miR-16 caused the S100β factor to turn on the expression of serotonergic functions in noradrenergic neurons[64].

Viral manipulation of specific miRNA levels was used in a study that investigated the role of miR-128b in fear extinction specifically in the infralimbic prefrontal cortex[95]. Lentiviral overexpression and 'sponge' knockdown of miR-128b in the infralimbic prefrontal cortex facilitated and inhibited fear extinction in mice, respectively. The authors suggested several target genes of miR-128, which were validated in *in vitro* assays. Interestingly, the sequence encoding the pre-miR-128 precursor is embedded within an intron of *ARPP21* (cyclic AMP-regulated phosphoprotein, 21 kDa; also known as *RCS*), and the miR-128–*Arpp21* interaction potentially modulates fear extinction[95].

**In vitro *assays.*** The 'gold standard' for confirming an interaction between an miRNA and a putative target gene is an *in vitro* luciferase assay. To this end, the 3′ UTR of the mRNA of the target gene is sub-cloned downstream to a luciferase reporter gene. The construct is then introduced into cells with the miRNA that is predicted to target it, and the amount of light produced by the luciferase

Transgenerational effects
Phenotype changes in the offspring that are caused by parental exposure to environmental factors.

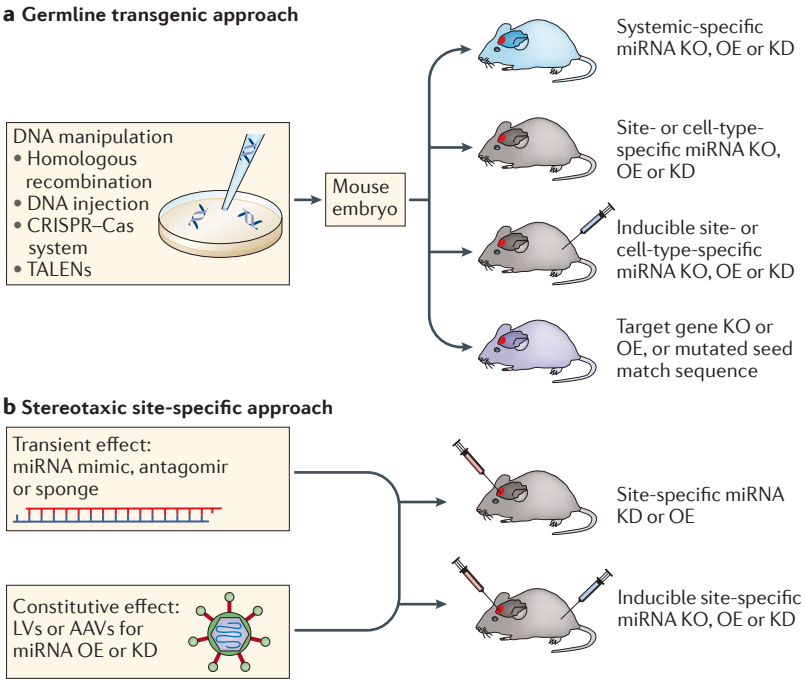



Figure 3 | **Approaches for manipulating specific brain microRNA expression levels and function. a** | Germline transgenic approaches include the generation of knockout (KO), overexpression (OE) or knockdown (KD) mouse lines for a specific microRNA (miRNA) or miRNA target gene. Such inheritable genetic manipulations are performed on mouse embryos using various approaches. Systemic manipulation of miRNA genes can be performed by generating, for example, a developmental KO mouse model; however, such models are associated with developmental compensatory changes and lack brain-area spatial specificity. These limitations can be overcome when using conditional KO mice for a specific miRNA that are generated using genetic systems such as Cre–*loxP*. In these models, for example, mice can be engineered to have a precursor miRNA (pre-miRNA) gene between *loxP* sites so that when they are crossbred with mice expressing Cre-recombinase under the control of a site- or cell-type-specific promoter, a conditional KO of that miRNA occurs only in the subset of cells expressing Cre-recombinase. To better control the temporal onset of the genetic manipulation, one can use inducible Cre-recombinase mouse lines or the Tet-ON–Tet-OFF systems. Alternatively, such approaches can be used to manipulate a protein-coding gene that is the target of a specific miRNA, thus providing information about the potential reciprocal interaction of the gene and miRNA. For example, establishing a transgenic mouse model that carries a mutation in a seed match sequence of a specific miRNA located in the 3′ untranslated region of the target transcript can provide *in vivo* proof of an interaction between the miRNA and the target gene. Germline transgenic approaches that use homologous recombination to 'knock in' a DNA fragment (through the injection of DNA into mouse embryos) are laborious, expensive and time-intensive; however, newer genome-engineering methods — such as CRISPR (clustered regularly interspaced short palindromic repeat)–Cas (CRISPR-associated protein) systems[112–114] or TALENs (transcription activator-like effector nucleases)[115–117] — can shorten this procedure. **b** | Stereotaxic site-specific approaches can be used to manipulate specific brain miRNAs in a process that involves either modified nucleic acids or recombinant viruses that induce, respectively, transient or constitutive effects on miRNA function. These approaches include intracerebroventricular or site-specific administration of oligonucleotide sequences that either overexpress the miRNA of interest (by introducing an miRNA mimic) or knock down the level of the relevant miRNA (by expressing a complementary 'antisense' oligonucleotide sequence of the mature miRNA (an 'antagomir')). Another alternative process, which blocks the effect of the miRNAs of interest, is the administration to the brain of oligonucleotide sequences containing multiple repeats of the miRNA-specific seed sequence ('sponge'). When constitutive changes in the expression levels of the miRNA are required, a viral approach is used and the relevant brain area is stereotactically injected with lentiviruses (LVs) or adeno-associated viruses (AAVs) that are designed to knock down or overexpress the specific miRNA. The pinnacle of miRNA manipulation is the combination of viral approaches with transgenic mouse models in order to obtain improved spatiotemporal CNS specificity[118].

enzyme catalysing its substrate is measured. A reduction in light output is interpreted as repression of luciferase activity that is induced by the miRNA. If mutating the seed match sequence in the 3′ UTR abolishes the repression, it can be concluded that direct miRNA–target interaction occurs *in vitro*. This conclusion can be confirmed by identifying a negative correlation between the level of an miRNA and its target gene in response to treatments *in vitro,* or by detecting a decrease or an increase in expression of the target gene when the miRNA is overexpressed or downregulated, respectively. Additionally, *in vitro* studies may be useful for in-depth investigations of, for example, the signalling pathways upstream or downstream of a specific miRNA in a cellular model for the pathology of interest. Alternatively, primary neuronal cultures from specific brain regions or cell types can be generated from miRNA-related rodent models for psychiatric disorders, such as mouse lines with altered levels of a specific miRNA. Such culture studies can help investigators to identify any alterations in molecular, physiological and structural properties of neurons in response to manipulation of miRNA pathways.

*In vitro* experiments in miRNA studies are often informative and are a relatively quick way to test results that are obtained using other methods (such as animal models, described above). However, one must remember that these are artificial, reductionist set-ups, and results obtained 'in the dish' should be validated in higher levels of analysis, especially if they pertain to complex disorders such as psychiatric disorders.

A study on the role of miRNAs in mediating stress-induced anxiety[81] provides an example of the use of *in vitro* methods (in addition to *in vivo* approaches). The study showed that, in adult mice, acute stress changed the miRNA expression profile in the amygdala; notably, the authors found a prominent upregulation in miR-34 expression. In a separate experiment, virus-mediated overexpression of miR-34 in the amygdala prevented stress-induced anxiety. *In vitro* luciferase reporter assays confirmed the bioinformatics-informed prediction that miR-34 interacts with corticotropin-releasing hormone receptor 1 (*Crfr1*) mRNA[81]. In a mouse neuroblastoma cell line that endogenously expresses CRFR1, transfection with a miR-34-overexpressing virus resulted in a decreased response of CRFR1 to its ligand CRF, suggesting that miR-34 functionally regulates the molecular machinery of the response to stress[81]. This miRNA–target interaction was recently confirmed[96].

A study on the role of miR-212 in cocaine intake[97] provides an example of using *in vitro* methods to analyse the signalling pathways downstream of an miRNA. First, an miRNA screen of rat dorsal striatum following prolonged cocaine access revealed upregulation of miR-212. Second, virus-mediated overexpression of miR-212 in the dorsal striatum *in vivo* decreased the motivation to consume cocaine, whereas administration of miR-212 antagonists had the opposite effect. Subsequent *in vitro* experiments in HEK (human embryonic kidney) cells demonstrated that miR-212 increases cAMP-responsive element-binding protein (CREB) signalling via RAF1 and the co-activator CREB-regulated transcription co-activator 1 (CRTC1; also

known as TORC1). Furthermore, virus-mediated overexpression of CRTC1 in rat striatum had effects on cocaine self-administration that were similar to those of miR-212 overexpression[97]. Thus, using *in vitro* tools along with *in vitro* manipulations provided comprehensive insights into the role of miR-212 in cocaine intake.

### Bioinformatics

Various bioinformatics tools are available that can be applied to miRNA data. These include algorithms for the prediction of miRNA–target gene interactions, comparative genomics data on miRNA conservations, tools for the discovery of new miRNAs and databases containing the results of miRNA expression-pattern screens. In some of the studies described above, bioinformatics was used to analyse results from high-throughput screens, mainly to generate target predictions for miRNAs that are differentially expressed in humans with a psychiatric disorder or in mouse or cellular models of these conditions. A less frequent but important use for bioinformatics is to predict which miRNAs might target protein-coding genes that have been associated with psychiatric disorders[64]. In addition, bioinformatics approaches have been used to assess whether polymorphisms[98,99] or CNVs[100,101] that have been associated with specific disorders influence the expression levels or activity of specific miRNA pathways. These *in silico* approaches are generally quick and cheap. However, one should keep in mind that algorithm-based predictions are error prone and may lead to both false-positive and false-negative results. Moreover, the predictions are algorithm specific, and the overlap in the results generated by different algorithms is often small. Therefore, the results obtained from bioinformatic approaches require 'wet' validations.

Bioinformatics approaches were used both to identify miRNA–target interactions associated with schizophrenia and to assign miR-185 targets to subcellular domains in the study mentioned above[94]. This study identified upregulation of an uncharacterized non-coding RNA (2310044H10Rik) in the brain of *Df(16)A*$^{+/-}$ mice. Bioinformatics analysis, which involves the cross-referencing of several prediction algorithms, identified miR-185, within the 22q11.2 microdeletion region, as a putative regulator of this transcript[94]. Subsequent *in vitro* experiments confirmed that there was a strong interaction between miR-185 and the transcript (which the authors named miRNA target of the 22q11.2 microdeletion (*Mirta22*))[94]. *Mirta22* is neuron specific and is enriched in Golgi-related structures, similarly to several other miR-185 putative targets, as revealed by bioinformatic functional annotation clustering analysis[94]. Dysregulation of Golgi-related genes can affect subcellular trafficking and secretion, which is necessary for the establishment and maintenance of neuronal connections. Spine and dendrite formation were indeed disrupted in *Df(16)A*$^{+/-}$ mice, and this effect could be reversed by overexpression of miR-185 or downregulation of Mirta22 levels[94]. Thus, combining genetic, *in vivo* and *in vitro* experiments with bioinformatics analysis has provided new insights into the pathophysiology of this syndromic form of schizophrenia.

### Open questions and future directions

This Review of the first era of research on the role of miRNAs in psychiatric disorders has identified several subjects that require further study. First, not much is known regarding the molecular mechanisms mediating the changes in miRNA levels observed in models of these disorders. This contrasts with the situation in, for example, cancer research, in which altered miRNA promoter methylation patterns have been found to explain some of the modified expression patterns of miRNAs associated with cancers (reviewed in REF. 102).

Second, many studies are limited to identifying one miRNA–target interaction in a single brain site in a specific disorder. Extending studies to other brain areas and neuronal circuits, to multiple target genes and to the molecular pathways associated with the miRNA of interest will provide more insights into the role of the miRNA in the disorder.

Third, there is a need for a comprehensive map of the spatiotemporal expression profile of endogenous miRNAs in the developing and adult brain, in specific cell types, in subcellular fractions (such as in synaptosomes), in health and in various psychiatric conditions. Such knowledge is crucial to expand our understanding of the possible behavioural and physiological functions of these miRNAs. Some systematic efforts have been made to profile miRNA expression patterns in humans, mice and other organisms across tissues (see the Swiss Institute of Bioinformatics' smirnaDB database, for example), in the developing human[103] and zebrafish[104] brain, and in part of the adult mouse brain[67]. Creating an atlas of mouse and human miRNA expression profiles equivalent to the mRNA Allen Brain Atlas would be very informative.

Fourth, there is evidence that miRNAs can mediate the effects of prenatal stress on the sexually dimorphic organization of the brain[105]. This suggests that research is needed to explore the role of miRNAs in sex differences in normal and pathological behaviours, as well as their possible involvement in mediating the sex differences in the prevalence of several psychiatric diseases (such as increased risk for depression and anxiety in women, and for autism in men). Another area of interest that is relatively unexplored concerns the possible involvement of miRNAs in mediating individual differences related to resiliency or susceptibility to psychiatric disorders[83].

Many studies measure the effects of miRNAs on the expression of their target gene at the transcript level only or use methods with low sensitivity to small changes in protein levels, such as western blotting. Using a more sensitive and broader analysis method, such as mass spectrometry proteomics[106], can provide a wider view on the role of specific miRNAs in the regulation of the functional form of their multiple target genes.

The roles of miRNAs in several types of psychiatric disorders have not been examined yet. We did not find any reports on the role of miRNAs in eating disorders, sexual dysfunction, somatic symptoms or personality disorders — perhaps this situation is due to the relatively limited animal models for some these conditions.

The availability of high-throughput methods has flooded the literature with lists of miRNAs that are altered in various models of psychiatric disorders. There is often little overlap between the results of studies from different laboratories, suggesting that systematic meta-analyses are much needed. Such comparisons may identify which miRNAs are associated with specific disorders. Some specific miRNAs are found again and again in screen results from models of different disorders. This suggests that these miRNAs might play a part in basic neuronal functions that are impaired in several disorders, such as synaptic plasticity. Alternatively, such miRNAs might be part of an endophenotype that is common to several disorders. Combining systems biology and computational approaches with experimental 'wet' studies can help to reveal such miRNAs.

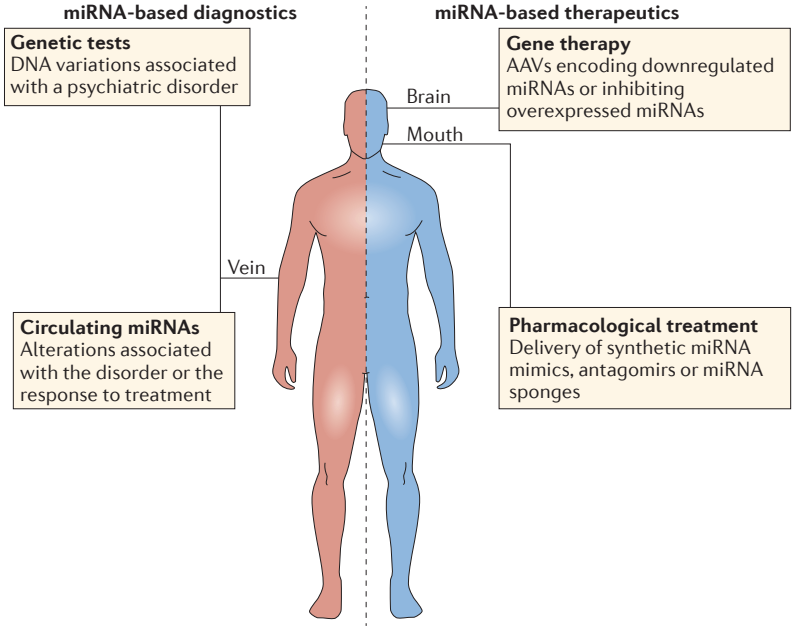



Figure 4 | **Using microRNA biology in psychiatry for diagnostics or therapeutics.** MicroRNA (miRNA)-related analysis might be used as non-invasive biomarkers in psychiatry for diagnostic purposes. Genetic analysis of single-nucleotide polymorphisms (SNPs) or chromosomal modifications, such as DNA duplication or deletion, in miRNA-related genes that are associated with a psychiatric disorder could be used for diagnostics, as well as the selection and adjustment of treatments. In addition, levels of specific miRNAs could be measured in the circulating fluids, such as in different fractions of the blood, and used for diagnosis and monitoring of response to treatment. Manipulating specific miRNA levels might have potential in the treatment of psychiatric disorders. Viral vectors such as adeno-associated viruses (AAVs) could be used to overexpress or knock down a specific miRNA. Alternatively, drugs containing synthetic miRNA mimics or oligonucleotides designed to reduce the level of a specific miRNA (such as antagomirs or miRNA sponges) could be developed.

## Concluding remarks

miRNAs are emerging as pivotal modulators of normal and pathological behaviours. The fact that more than half of the protein-coding genes are predicted to be regulated by miRNAs and that each miRNA can regulate hundreds of different genes positions these molecules as possible 'master regulators' of many cellular processes. Furthermore, an miRNA can regulate the expression of several genes within a specific biological or cellular pathway. These unique features, together with rapidly increasing experimental data, are encouraging scientists to study the role of miRNAs in the regulation of different psychopathologies. Additionally, the possible involvement of miRNAs in different psychiatric disorders is further supported by the established role of miRNAs in brain development, the fact that some psychiatric disorders have a neurodevelopmental origin and the realization that most psychopathologies probably involve mutations in multiple genes.

The accumulating evidence presented in this Review suggests that miRNAs may function through several mechanisms to regulate behaviour. The expression level of some miRNAs changes following certain behavioural or pharmacological challenges, thus facilitating a subsequent change in the expression of target genes, which are putatively needed in order to direct certain behavioural outcomes. It is also possible that miRNAs can serve as 'buffers' to keep levels of their protein targets stable and to avoid them being upregulated to pathological levels in response to a challenge. Shedding light on the role of miRNAs in psychopathologies could lead to a better understanding of the molecular pathways disrupted in these disorders and possibly promote the much needed development of new therapeutic and diagnostic approaches (FIG. 4).


1. Lupien, S. J., McEwen, B. S., Gunnar, M. R. & Heim, C. Effects of stress throughout the lifespan on the brain, behaviour and cognition. *Nature Rev. Neurosci.* **10**, 434–445 (2009).
2. Kendler, K. S., Gatz, M., Gardner, C. O. & Pedersen, N. L. A Swedish national twin study of lifetime major depression. *Am. J. Psychiatry* **163**, 109–114 (2006).
3. Sullivan, P. F., Neale, M. C. & Kendler, K. S. Genetic epidemiology of major depression: review and meta-analysis. *Am. J. Psychiatry* **157**, 1552–1562 (2000).
4. Levinson, D. F. *et al.* Genetic studies of major depressive disorder: why are there no genome-wide association study findings and what can we do about it? *Biol. Psychiatry* **76**, 510–512 (2014).
5. Schizophrenia Working Group of the Psychiatric Genomics Consortium. Biological insights from 108 schizophrenia-associated genetic loci. *Nature* **511**, 421–427 (2014).
6. Kendler, K. S., Karkowski, L. M. & Prescott, C. A. Causal relationship between stressful life events and the onset of major depression. *Am. J. Psychiatry* **156**, 837–841 (1999).
7. Kessler, R. C., Davis, C. G. & Kendler, K. S. Childhood adversity and adult psychiatric disorder in the US National Comorbidity Survey. *Psychol. Med.* **27**, 1101–1119 (1997).
8. Molnar, B. E., Buka, S. L. & Kessler, R. C. Child sexual abuse and subsequent psychopathology: results from the National Comorbidity Survey. *Am. J. Public Health* **91**, 753–760 (2001).
9. Kessler, R. C. *et al.* Childhood adversities and adult psychopathology in the WHO World Mental Health Surveys. *Br. J. Psychiatry* **197**, 378–385 (2010).
10. Bale, T. L. *et al.* Early life programming and neurodevelopmental disorders. *Biol. Psychiatry* **68**, 314–319 (2010).
11. Boersma, G. J. *et al.* Long-term impact of early life events on physiology and behaviour. *J. Neuroendocrinol.* **26**, 587–602 (2014).
12. Provençal, N. & Binder, E. B. The effects of early life stress on the epigenome: from the womb to adulthood and even before. *Exp. Neurol.* http://dx.doi.org/10.1016/j.expneurol.2014.09.001 (2014).
13. Cortijo, S. *et al.* Mapping the epigenetic basis of complex traits. *Science* **343**, 1145–1148 (2014).
14. Jirtle, R. L. & Skinner, M. K. Environmental epigenomics and disease susceptibility. *Nature Rev. Genet.* **8**, 253–262 (2007).
15. Bartel, D. P. MicroRNAs: target recognition and regulatory functions. *Cell* **136**, 215–233 (2009).
**This highly cited review describes the basic miRNA–target gene seed–seed match interaction and the common modes of regulation by miRNAs.**
16. Friedman, R. C., Farh, K. K., Burge, C. B. & Bartel, D. P. Most mammalian mRNAs are conserved targets of microRNAs. *Genome Res.* **19**, 92–105 (2009).
17. Kim, V. N., Han, J. & Siomi, M. C. Biogenesis of small RNAs in animals. *Nature Rev. Mol. Cell Biol.* **10**, 126–139 (2009).
**This review is recommended for further reading on the biogenesis of miRNA.**
18. Carthew, R. W. & Sontheimer, E. J. Origins and mechanisms of miRNAs and siRNAs. *Cell* **136**, 642–655 (2009).

19. Krol, J., Loedige, I. & Filipowicz, W. The widespread regulation of microRNA biogenesis, function and decay. *Nature Rev. Genet.* **11**, 597–610 (2010).
20. Sun, K. & Lai, E. C. Adult-specific functions of animal microRNAs. *Nature Rev. Genet.* **14**, 535–548 (2013).
21. Sun, A. X., Crabtree, G. R. & Yoo, A. S. MicroRNAs: regulators of neuronal fate. *Curr. Opin. Cell Biol.* **25**, 215–221 (2013).
22. Bushati, N. & Cohen, S. M. MicroRNAs in neurodegeneration. *Curr. Opin. Neurobiol.* **18**, 292–296 (2008).
23. Eacker, S. M., Dawson, T. M. & Dawson, V. L. Understanding microRNAs in neurodegeneration. *Nature Rev. Neurosci.* **10**, 837–841 (2009).
24. Salta, E. & De Strooper, B. Non-coding RNAs with essential roles in neurodegenerative disorders. *Lancet Neurol.* **11**, 189–200 (2012).
25. Schratt, G. microRNAs at the synapse. *Nature Rev. Neurosci.* **10**, 842–849 (2009).
26. Vo, N. K., Cambronne, X. A. & Goodman, R. H. MicroRNA pathways in neural development and plasticity. *Curr. Opin. Neurobiol.* **20**, 457–465 (2010).
27. Wang, W., Kwon, E. J. & Tsai, L. H. MicroRNAs in learning, memory, and neurological diseases. *Learn. Mem.* **19**, 359–368 (2012).
28. Thomson, D. W., Bracken, C. P. & Goodall, G. J. Experimental strategies for microRNA target identification. *Nucleic Acids Res.* **39**, 6845–6853 (2011).
29. Pritchard, C. C., Cheng, H. H. & Tewari, M. MicroRNA profiling: approaches and considerations. *Nature Rev. Genet.* **13**, 358–369 (2012).
**In this review, the different approaches for high-throughput screening of miRNA are critically compared.**
30. Hommers, L. G., Domschke, K. & Deckert, J. Heterogeneity and individuality: microRNAs in mental disorders. *J. Neural Transm.* **122**, 79–97 (2015).
31. Geaghan, M. & Cairns, M. J. MicroRNA and posttranscriptional dysregulation in psychiatry. *Biol. Psychiatry*. http://dx.doi.org/10.1016/j.biopsych.2014.12.009 (2014).
32. Ryan, B. M., Robles, A. I. & Harris, C. C. Genetic variation in microRNA networks: the implications for cancer research. *Nature Rev. Cancer* **10**, 389–402 (2010).
**This review discusses the potential effects of SNPs in miRNA genes on biological function using data from cancer research.**
33. Schizophrenia Psychiatric Genome-Wide Association Study Consortium. Genome-wide association study identifies five new schizophrenia loci. *Nature Genet.* **43**, 969–976 (2011).
34. International HapMap Consortium. The International HapMap Project. *Nature* **426**, 789–796 (2003).
35. Guella, I. *et al.* Analysis of miR-137 expression and *rs1625579* in dorsolateral prefrontal cortex. *J. Psychiatr. Res.* **47**, 1215–1221 (2013).
36. Szulwach, K. E. *et al.* Cross talk between microRNA and epigenetic regulation in adult neurogenesis. *J. Cell Biol.* **189**, 127–141 (2010).
37. Smrt, R. D. *et al.* MicroRNA miR-137 regulates neuronal maturation by targeting ubiquitin ligase mind bomb-1. *Stem Cells* **28**, 1060–1070 (2010).
38. Wright, C., Turner, J. A., Calhoun, V. D. & Perrone-Bizzozero, N. Potential impact of miR-137 and its targets in schizophrenia. *Front. Genet.* **4**, 58 (2013).
39. Guan, F. *et al. MIR137* gene and target gene *CACNA1C* of miR-137 contribute to schizophrenia susceptibility in Han Chinese. *Schizophr. Res.* **152**, 97–104 (2014).
40. Green, M. J. *et al.* Genome-wide supported variant *MIR137* and severe negative symptoms predict membership of an impaired cognitive subtype of schizophrenia. *Mol. Psychiatry* **18**, 774–780 (2013).
41. Cummings, E. *et al.* Mood congruent psychotic symptoms and specific cognitive deficits in carriers of the novel schizophrenia risk variant at *MIR-137*. *Neurosci. Lett.* **532**, 33–38 (2013).
42. Lett, T. A. *et al.* The genome-wide supported microRNA-137 variant predicts phenotypic heterogeneity within schizophrenia. *Mol. Psychiatry* **18**, 443–450 (2013).
43. Whalley, H. C. *et al.* Impact of a microRNA *MIR137* susceptibility variant on brain function in people at high genetic risk of schizophrenia or bipolar disorder. *Neuropsychopharmacology* **37**, 2720–2729 (2012).
44. Mothersill, O. *et al.* Effects of *MIR137* on fronto-amygdala functional connectivity. *Neuroimage* **90**, 189–195 (2014).
45. Liu, B. *et al.* The impact of *MIR137* on dorsolateral prefrontal-hippocampal functional connectivity in healthy subjects. *Neuropsychopharmacology* **39**, 2153–2160 (2014).
46. Collins, A. L. *et al.* Transcriptional targets of the schizophrenia risk gene *MIR137*. *Transl. Psychiatry* **4**, e404 (2014).
47. Hill, M. J. *et al.* Transcriptional consequences of schizophrenia candidate miR-137 manipulation in human neural progenitor cells. *Schizophr. Res.* **153**, 225–230 (2014).
48. Kwon, E., Wang, W. & Tsai, L. H. Validation of schizophrenia-associated genes *CSMD1*, *C10orf26*, *CACNA1C* and *TCF4* as miR-137 targets. *Mol. Psychiatry* **18**, 11–12 (2013).
49. Kim, A. H. *et al.* Experimental validation of candidate schizophrenia gene *ZNF804A* as target for hsa-miR-137. *Schizophr. Res.* **141**, 60–64 (2012).
50. Devanna, P. & Vernes, S. C. A direct molecular link between the autism candidate gene *RORa* and the schizophrenia candidate *MIR137*. *Sci. Rep.* **4**, 3994 (2014).
51. Kai, Z. S. & Pasquinelli, A. E. MicroRNA assassins: factors that regulate the disappearance of miRNAs. *Nature Struct. Mol. Biol.* **17**, 5–10 (2010).
52. Lopez, J. P. *et al.* miR-1202 is a primate-specific and brain-enriched microRNA involved in major depression and antidepressant treatment. *Nature Med.* **20**, 764–768 (2014).
**In this study, a specific miRNA was linked to human depression and treatment response by using both post-mortem tissue and circulating miRNA analysis.**
53. Issler, O. *et al.* MicroRNA 135 is essential for chronic stress resiliency, antidepressant efficacy, and intact serotonergic activity. *Neuron* **83**, 344–360 (2014).
54. Nestler, E. J. & Hyman, S. E. Animal models of neuropsychiatric disorders. *Nature Neurosci.* **13**, 1161–1169 (2010).
55. Kosaka, N. *et al.* Secretory mechanisms and intercellular transfer of microRNAs in living cells. *J. Biol. Chem.* **285**, 17442–17452 (2010).
56. Wang, K., Zhang, S., Weber, J., Baxter, D. & Galas, D. J. Export of microRNAs and microRNA-protective protein by mammalian cells. *Nucleic Acids Res.* **38**, 7248–7259 (2010).
57. Vickers, K. C., Palmisano, B. T., Shoucri, B. M., Shamburek, R. D. & Remaley, A. T. MicroRNAs are transported in plasma and delivered to recipient cells by high-density lipoproteins. *Nature Cell Biol.* **13**, 423–433 (2011).
58. Cortez, M. A. *et al.* MicroRNAs in body fluids — the mix of hormones and biomarkers. *Nature Rev. Clin. Oncol.* **8**, 467–477 (2011).
**This review is recommended for further reading on the theory and practice of using circulating miRNA as a disease biomarker.**
59. Redis, R. S., Calin, S., Yang, Y., You, M. J. & Calin, G. A. Cell-to-cell miRNA transfer: from body homeostasis to therapy. *Pharmacol. Ther.* **136**, 169–174 (2012).
60. Chen, X., Liang, H., Zhang, J., Zen, K. & Zhang, C.-Y. Horizontal transfer of microRNAs: molecular mechanisms and clinical applications. *Protein Cell* **3**, 28–37 (2012).
61. Schwarzenbach, H., Hoon, D. S. & Pantel, K. Cell-free nucleic acids as biomarkers in cancer patients. *Nature Rev. Cancer* **11**, 426–437 (2011).
62. Guay, C. & Regazzi, R. Circulating microRNAs as novel biomarkers for diabetes mellitus. *Nature Rev. Endocrinol.* **9**, 513–521 (2013).
63. Rao, P., Benito, E. & Fischer, A. MicroRNAs as biomarkers for CNS disease. *Front. Mol. Neurosci.* **6**, 39 (2013).
64. Baudry, A., Mouillet-Richard, S., Schneider, B., Launay, J. M. & Kellermann, O. miR-16 targets the serotonin transporter: a new facet for adaptive responses to antidepressants. *Science* **329**, 1537–1541 (2010).
**In this comprehensive study, a specific miRNA was strongly linked to the mode of action of SSRIs using animal and cellular models.**
65. Mouillet-Richard, S., Baudry, A., Launay, J. M. & Kellermann, O. MicroRNAs and depression. *Neurobiol. Dis.* **46**, 272–278 (2011).
66. O'Connor, R. M., Dinan, T. G. & Cryan, J. F. Little things on which happiness depends: microRNAs as novel therapeutic targets for the treatment of anxiety and depression. *Mol. Psychiatry* **17**, 359–376 (2012).
67. He, M. *et al.* Cell-type-based analysis of microRNA profiles in the mouse brain. *Neuron* **73**, 35–48 (2012).
68. Volk, N. *et al.* MicroRNA-19b associates with Ago2 in the amygdala following chronic stress and regulates the adrenergic receptor β1. *J. Neurosci.* **34**, 15070–15082 (2014).
69. Hausser, J. & Zavolan, M. Identification and consequences of miRNA–target interactions — beyond repression of gene expression. *Nature Rev. Genet.* **15**, 599–612 (2014).
70. Wu, H. *et al.* Genome-wide analysis reveals methyl-CpG-binding protein 2-dependent regulation of microRNAs in a mouse model of Rett syndrome. *Proc. Natl Acad. Sci. USA* **107**, 18161–18166 (2010).
71. Chahrour, M. & Zoghbi, H. Y. The story of Rett syndrome: from clinic to neurobiology. *Neuron* **56**, 422–437 (2007).
72. Klein, M. E. *et al.* Homeostatic regulation of MeCP2 expression by a CREB-induced microRNA. *Nature Neurosci.* **10**, 1513–1514 (2007).
73. Gapp, K., von Ziegler, L., Tweedie-Cullen, R. Y. & Mansuy, I. M. Early life epigenetic programming and transmission of stress-induced traits in mammals: how and when can environmental factors influence traits and their transgenerational inheritance? *Bioessays* **36**, 491–502 (2014).
74. Rodgers, A. B., Morgan, C. P., Bronson, S. L., Revello, S. & Bale, T. L. Paternal stress exposure alters sperm microRNA content and reprograms offspring HPA stress axis regulation. *J. Neurosci.* **33**, 9003–9012 (2013).
**In this paper, it is implied that sperm miRNAs are mediators of the transgenerational effects of stress.**
75. Gapp, K. *et al.* Implication of sperm RNAs in transgenerational inheritance of the effects of early trauma in mice. *Nature Neurosci.* **17**, 667–669 (2014).
**Here it is shown that manipulating sperm RNAs enables the transgenerational transfer of a stress-induced phenotype.**
76. Kim, J. *et al.* A microRNA feedback circuit in midbrain dopamine neurons. *Science* **317**, 1220–1224 (2007).
77. Davis, T. H. *et al.* Conditional loss of Dicer disrupts cellular and tissue morphogenesis in the cortex and hippocampus. *J. Neurosci.* **28**, 4322–4330 (2008).
78. Hebert, S. S. *et al.* Genetic ablation of Dicer in adult forebrain neurons results in abnormal tau hyperphosphorylation and neurodegeneration. *Hum. Mol. Genet.* **19**, 3959–3969 (2010).
79. Schaefer, A. *et al.* Cerebellar neurodegeneration in the absence of microRNAs. *J. Exp. Med.* **204**, 1553–1558 (2007).
80. Tao, J. *et al.* Deletion of astroglial Dicer causes non-cell-autonomous neuronal dysfunction and degeneration. *J. Neurosci.* **31**, 8306–8319 (2011).
81. Haramati, S. *et al.* MicroRNA as repressors of stress-induced anxiety: the case of amygdalar miR-34. *J. Neurosci.* **31**, 14191–14203 (2011).
82. Konopka, W. *et al.* MicroRNA loss enhances learning and memory in mice. *J. Neurosci.* **30**, 14835–14842 (2010).
83. Dias, C. *et al.* β-catenin mediates stress resilience through Dicer1/microRNA regulation. *Nature* **516**, 51–55 (2014).
**In this comprehensive study, resiliency to chronic stress was linked to increased *Dicer* expression, implying a prominent role for miRNAs in mediating resiliency.**
84. Karayiorgou, M. *et al.* Schizophrenia susceptibility associated with interstitial deletions of chromosome 22q11. *Proc. Natl Acad. Sci. USA* **92**, 7612–7616 (1995).
85. Stark, K. L. *et al.* Altered brain microRNA biogenesis contributes to phenotypic deficits in a 22q11-deletion mouse model. *Nature Genet.* **40**, 751–760 (2008).
**This is the first study linking the 22q11 deletion associated with schizophrenia with aberrant miRNA biogenesis.**
86. Perkins, D. O. *et al.* microRNA expression in the prefrontal cortex of individuals with schizophrenia and schizoaffective disorder. *Genome Biol.* **8**, R27 (2007).
87. Santarelli, D. M., Beveridge, N. J., Tooney, P. A. & Cairns, M. J. Upregulation of dicer and microRNA expression in the dorsolateral prefrontal cortex Brodmann area 46 in schizophrenia. *Biol. Psychiatry* **69**, 180–187 (2011).
88. Beveridge, N. J., Gardiner, E., Carroll, A. P., Tooney, P. A. & Cairns, M. J. Schizophrenia is associated with an increase in cortical microRNA biogenesis. *Mol. Psychiatry* **15**, 1176–1189 (2010).
89. Manolio, T. A. *et al.* Finding the missing heritability of complex diseases. *Nature* **461**, 747–753 (2009).

90. Earls, L. R. *et al.* Age-dependent microRNA control of synaptic plasticity in 22q11 deletion syndrome and schizophrenia. *J. Neurosci.* **32**, 14132–14144 (2012).
91. Ouchi, Y. *et al.* Reduced adult hippocampal neurogenesis and working memory deficits in the *Dgcr8*-deficient mouse model of 22q11.2 deletion-associated schizophrenia can be rescued by IGF2. *J. Neurosci.* **33**, 9408–9419 (2013).
92. Fenelon, K. *et al.* 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. USA* **108**, 4447–4452 (2011).
93. Schofield, C. M. *et al.* 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 (2011).
94. Xu, B., Hsu, P. K., Stark, K. L., Karayiorgou, M. & Gogos, J. A. Derepression of a neuronal inhibitor due to miRNA dysregulation in a schizophrenia-related microdeletion. *Cell* **152**, 262–275 (2013).
**Here, a specific miRNA gene in the 22q11 deletion is linked to its target gene, the dysregulation of which accounts for some of the symptoms of schizophrenia.**
95. Lin, Q. *et al.* The brain-specific microRNA miR-128b regulates the formation of fear-extinction memory. *Nature Neurosci.* **14**, 1115–1117 (2011).
96. Dias, B. G. *et al.* Amygdala-dependent fear memory consolidation via miR-34a and Notch signaling. *Neuron* **83**, 906–918 (2014).
97. Hollander, J. A. *et al.* Striatal microRNA controls cocaine intake through CREB signalling. *Nature* **466**, 197–202 (2010).
**In this paper, a specific miRNA in a specific brain site is linked to cocaine addiction, and the molecular mechanism of this interaction is comprehensively elucidated.**
98. Vaishnavi, V., Manikandan, M. & Munirajan, A. K. Mining the 3′UTR of autism-implicated genes for SNPs perturbing microRNA regulation. *Genom. Proteom. Bioinformat.* **12**, 92–104 (2014).
99. Gong, Y. *et al.* Polymorphisms in microRNA target sites influence susceptibility to schizophrenia by altering the binding of miRNAs to their targets. *Eur. Neuropsychopharmacol.* **23**, 1182–1189 (2013).
100. Vaishnavi, V., Manikandan, M., Tiwary, B. K. & Munirajan, A. K. Insights on the functional impact of microRNAs present in autism-associated copy number variants. *PLoS ONE* **8**, e56781 (2013).
101. Warnica, W. *et al.* Copy number variable microRNAs in schizophrenia and their neurodevelopmental gene targets. *Biol. Psychiatry* **77**, 158–166 (2015).
102. Esteller, M. Non-coding RNAs in human disease. *Nature Rev. Genet.* **12**, 861–874 (2011).
**This review discusses how miRNAs are potentially involved in disease aetiology and how they can be targeted with future therapeutics.**
103. Ziats, M. N. & Rennert, O. M. Identification of differentially expressed microRNAs across the developing human brain. *Mol. Psychiatry* **19**, 848–852 (2014).
104. Chen, P. Y. *et al.* The developmental miRNA profiles of zebrafish as determined by small RNA cloning. *Genes Dev.* **19**, 1288–1293 (2005).
105. Morgan, C. P. & Bale, T. L. Early prenatal stress epigenetically programs dysmasculinization in second-generation offspring via the paternal lineage. *J. Neurosci.* **31**, 11748–11755 (2011).
106. Breker, M. & Schuldiner, M. The emergence of proteome-wide technologies: systematic analysis of proteins comes of age. *Nature Rev. Mol. Cell Biol.* **15**, 453–464 (2014).
107. Borchert, G. M., Lanier, W. & Davidson, B. L. RNA polymerase III transcribes human microRNAs. *Nature Struct. Mol. Biol.* **13**, 1097–1101 (2006).
108. Lee, Y. *et al.* The nuclear RNase III Drosha initiates microRNA processing. *Nature* **425**, 415–419 (2003).
109. Yi, R., Qin, Y., Macara, I. G. & Cullen, B. R. Exportin-5 mediates the nuclear export of pre-microRNAs and short hairpin RNAs. *Genes Dev.* **17**, 3011–3016 (2003).
110. Chendrimada, T. P. *et al.* TRBP recruits the Dicer complex to Ago2 for microRNA processing and gene silencing. *Nature* **436**, 740–744 (2005).
111. Ambros, V. *et al.* A uniform system for microRNA annotation. *RNA* **9**, 277–279 (2003).
112. Wang, H. *et al.* One-step generation of mice carrying mutations in multiple genes by CRISPR/Cas-mediated genome engineering. *Cell* **153**, 910–918 (2013).
113. Mali, P. *et al.* RNA-guided human genome engineering via Cas9. *Science* **339**, 823–826 (2013).
114. Cong, L. *et al.* Multiplex genome engineering using CRISPR/Cas systems. *Science* **339**, 819–823 (2013).
115. Cui, X. *et al.* Targeted integration in rat and mouse embryos with zinc-finger nucleases. *Nature Biotech.* **29**, 64–67 (2011).
116. Bogdanove, A. J. & Voytas, D. F. TAL effectors: customizable proteins for DNA targeting. *Science* **333**, 1843–1846 (2011).
117. Wang, H. *et al.* TALEN-mediated editing of the mouse Y chromosome. *Nature Biotech.* **31**, 530–532 (2013).
118. Walz, W. (ed.) *Viral Vector Approaches In Neurobiology And Brain Diseases* (Springer, 2014).
119. Gao, J. *et al.* A novel pathway regulates memory and plasticity via SIRT1 and miR-134. *Nature* **466**, 1105–1109 (2010).
120. Griggs, E. M., Young, E. J., Rumbaugh, G. & Miller, C. A. MicroRNA-182 regulates amygdala-dependent memory formation. *J. Neurosci.* **33**, 1734–1740 (2013).
121. Im, H. I., Hollander, J. A., Bali, P. & Kenny, P. J. MeCP2 controls BDNF expression and cocaine intake through homeostatic interactions with microRNA-212. *Nature Neurosci.* **13**, 1120–1127 (2010).
122. Kocerha, J. *et al.* MicroRNA-219 modulates NMDA receptor-mediated neurobehavioral dysfunction. *Proc. Natl Acad. Sci. USA* **106**, 3507–3512 (2009).
123. Chandrasekar, V. & Dreyer, J. L. microRNAs miR-124, let-7d and miR-181a regulate cocaine-induced plasticity. *Mol. Cell Neurosci.* **42**, 350–362 (2009).
124. Chandrasekar, V. & Dreyer, J. L. Regulation of miR-124, let-7d, and miR-181a in the accumbens affects the expression, extinction, and reinstatement of cocaine-induced conditioned place preference. *Neuropsychopharmacology* **36**, 1149–1164 (2011).



### Acknowledgements
The authors acknowledge those whose work they could not cite owing to lack of space. They thank S. Haramati-Rodrig and O. Furman for their critical scientific editing; N. Volk for assisting with Box 1; and E. Geron, Y. Kuperman, Y. Ben-Efraim and N. Volk for reading the Review and providing comments. The authors also thank E. Hornstein for discussions and insights. A.C.'s work is supported by an FP7 grant from the European Research Council (260463); the Max Planck Society — Weizmann Institute of Science Laboratory for Experimental Neuropsychiatry and Behavioral Neurogenetics; a research grant from the Israel Science Foundation (803/11); research support from Roberto and Renata Ruhman; the Nella and Leon Benoziyo Center for Neurological Diseases; the Henry Chanoch Krenter Institute for Biomedical Imaging and Genomics; the Perlman Family Foundation founded by Louis L. and Anita M. Perlman for research in the field of metabolism; the Adelis Foundation; Marc Besen and the Pratt Foundation; and the Irving I. Moskowitz Foundation.


### Competing interests statement
The authors declare no competing interests.

### DATABASES
**Allen Brain Atlas:** http://www.brain-map.org
**miRBase nomenclature guide:** http://www.mirbase.org/help/nomenclature.shtml
**Pathway Interaction Database:** http://pid.nci.nih.gov
**smirnaDB:** http://www.mirz.unibas.ch/cloningprofiles/

**ALL LINKS ARE ACTIVE IN THE ONLINE PDF**