Cellular and Molecular Life Sciences (2020) 77:4347–4364
https://doi.org/10.1007/s00018-020-03542-7

REVIEW

miRNA regulation of social and anxiety-related behaviour

Ramanathan Narayanan[1] · **Gerhard Schratt**[1]

Received: 19 November 2019 / Revised: 31 March 2020 / Accepted: 27 April 2020 / Published online: 14 May 2020

Abstract

Neuropsychiatric disorders, including autism spectrum disorders (ASD) and anxiety disorders are characterized by a complex range of symptoms, including social behaviour and cognitive deficits, depression and repetitive behaviours. Although the mechanisms driving pathophysiology are complex and remain largely unknown, advances in the understanding of gene association and gene networks are providing significant clues to their aetiology. In recent years, small noncoding RNA molecules known as microRNA (miRNA) have emerged as a new gene regulatory layer in the pathophysiology of mental illness. These small RNAs can bind to the 3′-UTR of mRNA thereby negatively regulating gene expression at the post-transcriptional level. Their ability to regulate hundreds of target mRNAs simultaneously predestines them to control the activity of entire cellular pathways, with obvious implications for the regulation of complex processes such as animal behaviour. There is growing evidence to suggest that numerous miRNAs are dysregulated in pathophysiology of neuropsychiatric disorders, and there is strong genetic support for the association of miRNA genes and their targets with several of these conditions. This review attempts to cover the most relevant microRNAs for which an important contribution to the control of social and anxiety-related behaviour has been demonstrated by functional studies in animal models. In addition, it provides an overview of recent expression profiling and genetic association studies in human patient-derived samples in an attempt to highlight the most promising candidates for biomarker discovery and therapeutic intervention.

Keywords miRNA · Sociability · Anxiety · Behaviour

Abbreviations

AAV	Adeno associated virus
AChE	Acetyl cholinesterase
AGO	Argonaute
AMPAR	α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor
AS	Acute stress
ASD	Autism spectrum disorder
BChE	Butyrylcholinesterase
BD	Bipolar disorder
BDNF	Brain-derived neurotrophic factor
BLA	Basolateral amygdala
CA1	Cornu Ammonis area 1
Cas9	CRISPR associated protein 9
cAMP	Cyclic adenosine triphosphate
CCKBR	Cholecystokinin B receptor
CeA	Central amygdala
cGMP	Cyclic guanine triphosphate
CHMP2B	Charged multivesicular body protein 2b
circRNA	Circular RNA
CNR1	Cannabinoid receptor type 1
CNV	Copy number variation
CRF	Corticotrophin releasing factor
CRFR1	Corticotrophin releasing factor receptor 1
CRHR1	Corticotrophin releasing hormone receptor 1
CRISPR	Clustered regularly interspaced short palindromic repeats
CRS	Chronic restraint stress
DG	Dentate gyrus
DGCR8	DiGeorge syndrome chromosomal region 8
DRD2	Dopamine receptor D 2
DSM-V	Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition
Egr-1	Early growth response protein 1
EPAC	Exchange factor directly activated by cAMP
EPM	Elevated plus maze
Ezh2	Enhancer of zeste homolog 2

✉ Gerhard Schratt
Gerhard.schratt@hest.ethz.ch

[1] Lab of Systems Neuroscience, Department of Health Science and Technology, Institute for Neuroscience, Swiss Federal Institute of Technology ETH, Zurich, Switzerland

F1	Filial 1
Foxp2	Forkhead box protein P2
FTD	Frontotemporal dementia
GABA	γ-Aminobutyric acid
GABRA6	Gamma-aminobutyric acid receptor subunit alpha-6
GluA	Glutamate receptor, AMPA type
GluN2A	Glutamate receptor NMDA type 2A
GluR	Glutamate receptor
GR	Glucocorticoid receptor
GRIA	Glutamate receptor
GRM7	Metabotropic glutamate receptor 7
GWAS	Genome-wide association study
Hc	Hippocampus
hNSC	Human neural stem cell
HPA	Hypothalamus–pituitary–adrenal axis
5-HT2C	5-Hydroxytryptamine 2C
HTR2C	5-Hydroxytryptamine receptor 2C
ID	Intellectual disability
IKBKE	Inhibitor of nuclear factor kappa-B kinase subunit epsilon
IL-6	Interleukin-6
iPSC	Induced pluripotent stem cell
JAK	Janus kinase
KO	Knockout
lncRNA	Long non-coding RNA
LTD	Long term depression
LTP	Long term potentiation
MAOA	Monoamine oxidase A
MAP1B	Microtubule-associated protein 1B
MeCP2	Methyl CpG binding protein 2
mGluR	Metabotropic glutamate receptor
miRNA	Micro RNA
mPFC	Medial prefrontal cortex
mRNA	Messenger RNA
MSUS	Maternal separation, unexpected stress
ncRNA	Non-coding RNA
NMDA	*N*-Methyl-d-aspartate
OCD	Obsessive–compulsive disorder
PCR	Polymerase chain reaction
PD	Panic disorder
Pde10a	Phosphodiesterase 10A
PI3K	Phosphoinositide 3-kinase
piRNA	Piwi-interacting RNA
POMC	Pro-opiomelanocortin
Pre-miRNA	Precursor micro RNA
Pri-miRNA	Primary micro RNA
PSD95	Post-synaptic density 95
PTEN	Phosphatase and tensin homolog
PTSD	Post-traumatic stress disorder
PVN	Paraventricular nucleus
Rap1	Ras-related protein 1
RapGEFs	Rap guanine nucleotide exchange factor
RGS2	Regulator of G-protein signaling 2
RISC	RNA induced silencing complex
RNA	Ribonucleic acid
RNase	Ribonuclease
Sgk1	Serum and glucocorticoid-regulated kinase 1
Sirt1	Sirtuin 1
smFISH	Single-molecule fluorescent in situ hybridization
SNP	Single nucleotide polymorphism
STAT	Signal transducer and activator of transcription
TNF-a	Tumor necrosis factor alpha
TRBP	TAR RNA binding protein
USV	Ultrasonic vocalization
UTR	Untranslated region

Introduction

MicroRNAs (miRNAs) are a family of small non-coding RNAs (ncRNAs), which act at the post-transcriptional level to regulate gene expression. Ever since the discovery of the first miRNAs in *Caenorhabditis elegans, let-7 and lin-4* [1–3], numerous studies have been performed to explore the biogenesis pathways of these molecules (reviewed by [4, 5]). The majority of miRNAs are initially transcribed by RNA polymerase II as long primary transcripts (pri-miRNA) including a polyA tail and secondary hairpin structure(s). The 3′- and 5′-end of the pri-miRNA is then cleaved in the nucleus by the microprocessor complex, containing the RNase III family Drosha and DGCR8 as core components, to liberate a precursor miRNA (pre-miRNA), followed by its transport to the cytoplasm [6]. In the cytoplasm, another RNase III family enzyme Dicer together with the RNA binding protein TRBP then cleaves the hairpin structure of the pre-miRNA to form a miRNA duplex, in which two strands of about 22 nucleotides in length (guide/passenger strand, or 5-p/3-p-strand) are associated by Watson–Crick base pairing. This duplex is then dissociated upon loading of either the 5-p or 3-p strand, now called the mature miRNA into the miRNA-associated RNA-induced silencing complex (miRISC), which consists of one Argonaute family protein (AGO 1–4), along with accessory components such as Dicer and TRBP, among others [7].

MicroRNAs contain a 6–8 nucleotide long sequence at their 5′-end which is known as the "seed" sequence [8]. This sequence is important for target mRNA recognition since it is often fully complementary to a sequence in the 3′- untranslated region (3′-UTR) of the targeted mRNA, known as the "seed match". Once recruited to the target, miRISC suppresses protein production by inhibiting mRNA translation, promoting mRNA degradation, or both. In rare

cases when the miRNA is fully complementary to its target, the target mRNA is degraded upon miRISC recruitment by endonucleolytic cleavage catalysed by AGO2 [9, 10]. In addition to this canonical pathway, new non-canonical biogenesis pathways have been discovered which involve only Dicer but not the microprocessor complex [11]. However, the number of miRNAs which are produced through this pathway remains to be determined [12].

MiRNAs can originate in the genome from both the introns of protein-coding genes (host genes) [13] or regions without any known coding function [14]. MiRNAs can exist either singly or as clusters of up to several tens of different miRNA sequences [14–16]. Consequently, the expression levels of the individual miRNAs have a high correlation with the host gene expression [13]. The human genome contains 2656 annotated mature miRNAs (https://www.mirbase.org, release 22), and it is estimated that the expression of up to 50% of human genes is controlled by miRNAs [17]. This results from the fact that a single miRNA can regulate the expression of several hundred genes and that, on the other hand, the expression of a single gene may be affected by multiple miRNAs [18]. Besides this, the function of miRNA can be highly context-dependent, eliciting robust mRNA degradation in processes such as neuronal development and also subtle local changes in mRNA translation during synapse development [19, 20]. Due to their ability to regulate entire pathways, miRNAs are increasingly considered in the context of neuropsychiatric disorders as a potential diagnostic and therapeutic target.

Neuropsychiatric disorders refer to a group of diseases of the nervous system characterized by abnormalities in neuronal morphology, function, connectivity, which is manifested through distinct behavioural symptoms. These symptoms range from learning and memory deficits, intellectual disability, repetitive behaviours, impairments in social behaviour and anxiety, among others. The individual diseases though vary significantly in terms of the onset, brain region and circuits involved, but also often display overlapping symptoms and genetic architecture. In this review, we provide an overview of key findings that link specific miRNAs to the two spectra of neuropsychiatric disorders—autism spectrum disorder (ASD) and anxiety-related disorders.

Role of miRNAs in sociability—evidence from functional studies

miR379-410 cluster

The placental mammal-specific miR379-410 cluster consists of 38 miRNAs and displays paternal imprinting. Constitutive germline deletion of this cluster in mice results in a rather unusual combination of both hypersocial and anxiety-like behaviour, reminiscent of the rare neurodevelopmental disorders Williams and Angelman's syndrome [21]. Specifically, the knockout (KO) mice displayed significant increase in number of juvenile ultrasonic vocalizations (USVs) during reciprocal social interaction and increased social preference towards conspecific compared to an object in a three-chamber sociability test [22]. The knockout mice also displayed reduced repetitive behaviour as assessed through marble-burying test, together constituting an "anti-autistic" phenotype. These behavioural phenotypes were accompanied by increased synaptic spine density in the hippocampal CA1 pyramidal neurons and increased glutamatergic synapse-specific gene expression in the hippocampus of knockout mice as shown by RNA sequencing. Electrophysiological analysis also showed enhanced excitatory synaptic transmission, collectively signifying the role of this miRNA cluster in hippocampal excitatory transmission and sociability [22]. It should be noted, however, that changes in hippocampal synaptic transmission have not been causally linked to the "anti-autistic" phenotype in these mice. Future studies are needed to disentangle the contribution of specific neural circuits (e.g. amygdala–hippocampus–prefrontal cortex) to the observed behavioural phenotypes. Moreover, the contribution of each of the 38 miRNAs deleted in the model remains enigmatic. Bioinformatics analysis of hippocampal transcriptome data points to an exquisite function of a few "hub miRNAs", but these findings need to be experimentally validated. Notwithstanding, in light of a previous description of an autistic patient with a duplication of the miR379-410 genomic region [23], a more thorough examination of the therapeutic and diagnostic prospects of this miRNA cluster in ASD is clearly warranted.

miR-124

Frontotemporal dementia (FTD) is a late-onset disease resulting from degeneration and dysfunction of neuronal networks in the frontal and temporal lobes as well as subcortical regions, and is a leading cause of dementia after Alzheimer's disease. Studying FTD is also instructive in the context of social behaviour since a behavioural variant of FTD displays symptoms including social withdrawal, apathy and inappropriate repetitive behaviour [24]. One of the most abundantly expressed miRNAs in the brain, miR-124 is evolutionarily conserved and is implicated in several neurodevelopmental processes [25]. In a mouse model of frontotemporal dementia (FTD) in which a mutant version of the human CHMP2B gene is overexpressed in the forebrain (anterior part of the brain including the cerebral hemispheres, thalamus, hypothalamus, limbic system and olfactory bulb) neurons, reduced levels of miR-124 and increased expression of its direct targets GRIA2, GRIA3 and GRIA4 (encoding

the GluA2, 3 and 4 AMPAR subunits) was observed in the frontal cortex [26]. Adult CHMP2B transgenic mice (4 and 8 months old) displayed decreased sociability (as measured by the time mice spent interacting with each other), whereas social recognition and social memory were unaffected. Interestingly, intraperitoneal administration of an AMPAR antagonist and cortical GRIA2 silencing reversed this impairment in sociability. It is worth mentioning that the levels of miR-124 were found to decrease with age, and that the sociability defects were observed not before the age of 4 months, indicating that miR-124-dependent downregulation of GRIA2 might be particularly important to prevent a decline in sociability associated with ageing [26].

Interestingly, an earlier study also linked miR-124 to social behaviour. It was shown that the RapGEFs EPAC1/2, by stimulating the activity of the small Rho GTPase Rap1, inhibit miR-124 transcription through a regulatory element in the miR-124 promoter [27]. Consequently, the deletion of EPAC1/2 in mouse forebrain leads to an upregulation of miR-124, reduced levels of Egr-1 (Zif268), decreased synaptic plasticity and deficits in social behaviour. In addition, knockdown of miR-124 rescues the EPAC null phenotype [27]. Yet another recent study found that the silencing of miR-124a (miR-124-3p according to new nomenclature) in adult mouse dentate gyrus (DG) by a lentiviral approach resulted in autism-like phenotype as measured through marble-burying test, self-grooming and social interactions. This also resulted in increased levels of brain-derived neurotrophic factor (BDNF), a direct target of miR-124a. Interestingly, viral-mediated overexpression of BDNF in DG resulted in similar phenotypes [28].

Taken together, there are conflicting results as to whether interfering with miR-124 leads to a pro-social [27] or anti-social, ASD-like [26, 28] phenotype. The function of miR-124 in regulating social behaviour is therefore likely a result of the target spectrum that is dependent on the cellular context and/or developmental stage within the organism.

miR-137

A recent study investigated the effect of in vivo loss of function of miR-137, a miRNA implicated in several psychiatric conditions including autism, schizophrenia and bipolar disorder. While germline KO of miR-137 in mice results in postnatal lethality, heterozygous KO mice were viable [29]. Conditional deletion of miR-137 specifically in the developing nervous system using Nestin-Cre (cKO) resulted in synapse overgrowth as demonstrated by PSD95 and synaptophysin immunostaining. In addition, Golgi staining showed an increase in basal and apical spines in the hippocampal CA1 region, possibly pointing to deficits in synaptic pruning, which in turn leads to enhanced dendritic growth and complexity. The cKO mice displayed deficits in spatial learning and memory as assessed through Morris water maze and Barnes maze, which were paralleled by deficits in synaptic plasticity as assessed by electrophysiological LTP recordings in hippocampal slices. In addition, the cKO mice displayed increased repetitive behaviours as assessed through self-grooming and marble-burying test and increased anxiety in an open field test. Interestingly, the cKO mice also displayed a reduced social preference for a mouse over an object in a three-chamber test and no preference for a stranger mouse over a familiar mouse (social novelty). Subsequent proteomic, transcriptomic and bioinformatics analyses together with luciferase reporter assays identified Phosphodiesterase 10a (Pde10a) as one of the direct targets. Pde10a is highly expressed in the brain and regulates important signalling cascades by degrading the second messengers cAMP and cGMP. In a rescue experiment, papaverine, a Pde10a-specific inhibitor partially reduced the impairments in memory, social and repetitive behaviour. Similar results were obtained with the lentiviral knockdown of Pde10a in the mouse brain [29], further suggesting the pathophysiological relevance of this pathway in ASD. In addition, postsynaptic downregulation of miR-137 in hippocampal slices was shown to enhance AMPAR-mediated synaptic transmission and to interfere with mGluR-LTD [30], which could also play a role in the regulation of ASD-related behaviours.

In humans, 1p21.3 microdeletions affecting the MIR137 gene, among others, have been identified in individuals with ASD [31] and intellectual disability (ID) [32]. On the other hand, MIR137 was one of the top hits in a large GWAS for schizophrenia [33]. Subsequently, the associated SNP was shown to result in increased miR-137 levels, which suggests that, in contrast to ASD, miR-137 gain-of-function might contribute to the development of schizophrenia [34]. Surprisingly, follow-up functional studies in mice show that miR-137 overexpression in the dentate gyrus mainly affects cognitive performance (i.e. hippocampus-dependent learning) by altering presynaptic physiology, without notable effects on anxiety or risk-taking behaviour [34]. Thus, the bi-directional deviation from a physiological range of miR-137 expression leads to distinct neuropsychiatric conditions, presumably mediated by completely different cellular mechanisms. This will have to be taken into account if the restoration of miR-137 levels is considered as a therapeutic strategy in mental disease.

miR-17–92 cluster

Feingold syndrome is a rare neurodevelopmental condition characterized by microcephaly, facial dysmorphism, learning disabilities [35]. In an attempt to model this condition, a mouse model harbouring a heterozygous deletion of the miR-17-92 cluster was generated [36]. This miRNA cluster is mostly known for its role in oncogenesis but has recently

been shown to control neurogenesis both in the developing and adult brain [37, 38]. Interestingly, miR-17-92 +/− mice display reduced body growth and USVs during development. Behavioural phenotyping of adult mutant mice showed deficits in spatial memory as well as social novelty recognition. In addition, altered levels of dopamine and serotonin were found in the medial prefrontal cortex (mPFC) and hippocampus of the mutant mice [39]. Although mostly correlative, these data point to an important role of the miR-17-92 cluster in the development of neural circuits relevant for the control of social behaviour. Accordingly, SNPs within the cluster have been found to be overrepresented in ASD patients compared to controls [40]. Table 1 provides a summary of miRNAs implicated in social behaviour through functional studies in animal models. Figure 1 displays the corresponding brain regions involved and Fig. 2 provides an overview of the underlying molecular mechanisms.

Role of miRNAs in autism spectrum disorder—evidence from profiling and cellular studies

Autism spectrum disorders are a heterogeneous group of disorders characterized by deficits in social communication and restricted repetitive patterns of behaviour. ASD display high comorbidity with other neurological disorders, such as intellectual disability (ID), anxiety and/or epilepsy. ASD heritability was estimated to 50–60%, including both highly penetrant but rare genetic variants, chromosomal abnormalities and common variants with low penetrance [41, 42]. Given that epidemiologic studies indicate that ASD is mostly the result of the dysfunction of multiple gene networks rather than a single gene, posttranscriptional mechanisms such as miRNAs, which can alter entire gene networks, are increasingly studied in this context [43, 44].

Several studies have attempted to perform miRNA profiling in ASD patients in a range of tissues such as post mortem brain regions [45–48], peripheral blood [49], blood serum [50], lymphoblastoid cell lines [51–53], olfactory mucosal stem cells and primary skin fibroblasts [54]. There are a total of 156 unique miRNAs reported in these studies to be either upregulated or downregulated in ASD patients compared to healthy controls. Among these miRNAs, only 26 were identified by more than one study—seven overlapped in three studies while 19 in two studies. Even among these overlapping miRNAs, only 12 were reported to be deregulated in the same direction. Whereas eight of these 26 miRNAs were reported to change within the ASD cohort and control cohort with age [47], six were expressed at either low levels or undetectable in the dorsal frontal cortex of healthy human brains [55]. Another study using microarray profiling in a small group of Chinese patients with autism showed that a total of 77 miRNAs were differentially regulated compared to healthy controls. Subsequent quantitative reverse transcription-PCR analysis was used to validate that miR-557 and miR-486-3p were significantly increased in the majority of ASD patients [56]. A recent study extensively explored 527 mature miRNAs in the saliva of ASD patients through RNA sequencing. The results showed downregulation of five miRNAs (miR-28-3p, miR-148a-5p, miR-151a-3p, miR-125b-2-3p, miR-7706) and upregulation of four miRNAs (miR-665, miR-4705, miR-620, miR-1277-5p) in ASD group compared to typical development and non-autism developmental delay groups [57]. With the exception of miR-146a (see below), it should be noted that overlapping the different profiling studies did not yield a strong candidate microRNA to follow up in detailed mechanistic studies in cellular or animal models. The reasons are likely manifold, including but not limited to the low number of subjects investigated in the studies, the heterogeneous sources of the material and the different technologies used for the assessment of miRNA expression levels.

miR-146a

One of the miRNAs that appeared prominently from the human expression profiling studies is miR-146a. In rat primary cell culture, microarray analysis indicated that miR-146a was enriched in astrocytes compared to neurons and that the overexpression of miR-146a in neural stem cells drives astrocyte differentiation [58]. In addition, strong expression of miR-146a was observed in the mouse cortex, hippocampus and amygdala and miR-146a overexpression in mouse primary neuronal culture resulted in altered dendritic morphology, with a positive function of miR-146a in the regulation of proximal dendritic branching [54]. Similar results were obtained in human neural stem cells (hNSC), with miR-146a overexpression enhancing neurite outgrowth and branching as well as overall neuronal differentiation [59].

Further studies elucidate the role of miR-146a in neuronal function. In cultured mouse hippocampal neurons, inhibition of miR-146a leads to an increase of dendritic microtubule-associated protein 1B (MAP1B), thereby leading to AMPARs internalization and decreased synaptic transmission [60]. Interestingly, miR-146a overexpression in primary astrocyte culture resulted in a significant increase in uptake of extracellular glutamate, an abundant excitatory neurotransmitter in the brain, thereby potentially altering homeostasis and synaptic transmission [54].

Expression studies in human samples demonstrate that there is an increase in miR-146a levels during early childhood in the temporal lobe and patient-derived olfactory neural stem cells of ASD patients [54, 59]. Moreover, using small RNA sequencing, a study analysing miRNA

Table 1 List of miRNAs implicated in social behaviour through functional studies in animal models

miRNA	Animal model	Behavioural phenotype	Molecular mechanism	References	Reported in human studies
miR379-410 cluster	Constitutive germline deletion of the cluster in mice	Hypersocial behaviour, Increased ultrasonic vocalizations, Reduced repetitive behaviour, Increased anxiety-like behaviour	Increased excitatory and decreased inhibitory synapse-specific gene expression in hippocampus; Increased spine number, increased synaptic transmission in hippocampus	[22]	miR-494 [48, 49] miR-654-5p [54]
miR-124	Expression of mutant human CHMP2B gene in mouse forebrain neurons, Reduced levels of miR-124	Impaired social interaction	Increased expression of miR-124 targets GRIA2, GRIA3 and GRIA4 AMPAR subunits	[26]	
miR-124	Deletion of EPAC in mouse hippocampus, Increased levels of miR-124	Social behaviour deficits	Downregulation of Zif268 expression, decreased synaptic plasticity	[27]	
miR-124a (miR-124-3p)	Silencing of miR-124a (miR-124-3p) in mouse dentate gyrus by lentiviral approach	Impaired social interaction and increased repetitive behaviour	Increased levels of BDNF	[28]	
miR-137	Conditional deletion of miR-137 in the nervous system using Nestin-Cre	Deficits in spatial learning and memory, reduced social preference, increased repetitive behaviour, increased anxiety	Increased levels of Pde10a, deficits in synaptic pruning, and enhanced dendritic growth and complexity	[29]	
miR-17-92 cluster	Heterozygous deletion of the cluster	Reduced ultrasonic vocalizations, deficits in spatial memory, social novelty recognition	Altered levels of dopamine and serotonin in the mPFC and hippocampus	[39]	miR-92a-3p [49, 53] miR-19b-3p [48–50] miR-19a-3p [56] SNPs [40]

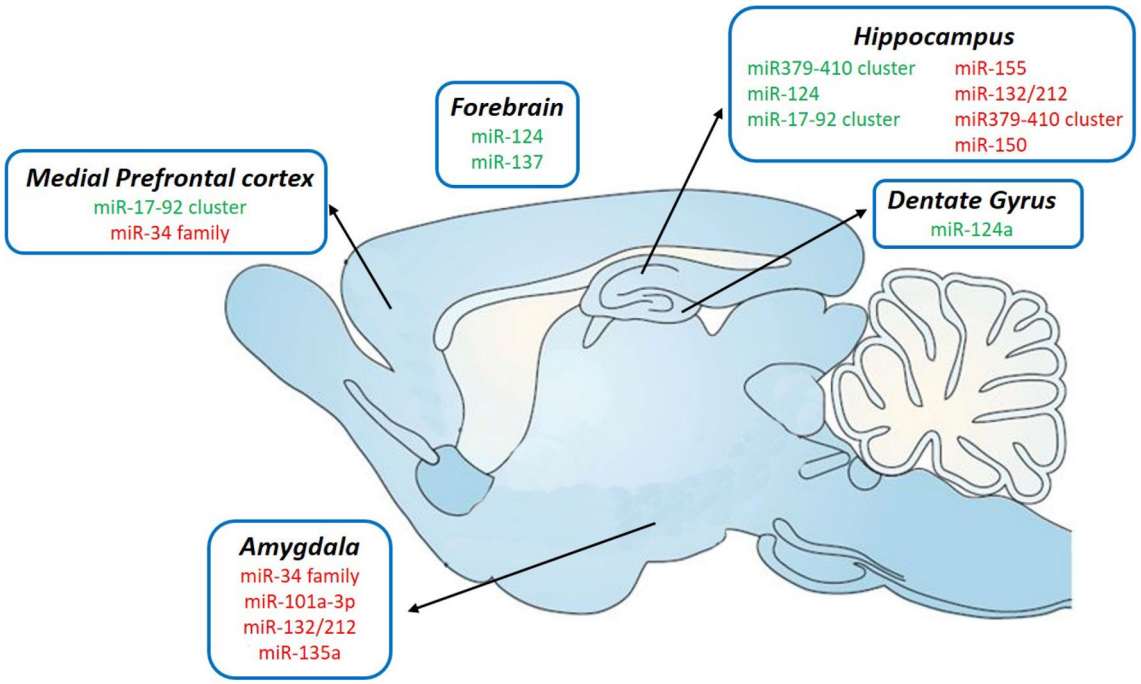

Fig. 1 Brain region-specific role of miRNAs in sociability and anxiety behaviors identified through functional studies in mouse models. miRNAs associated with sociability are marked in green and anxiety in red

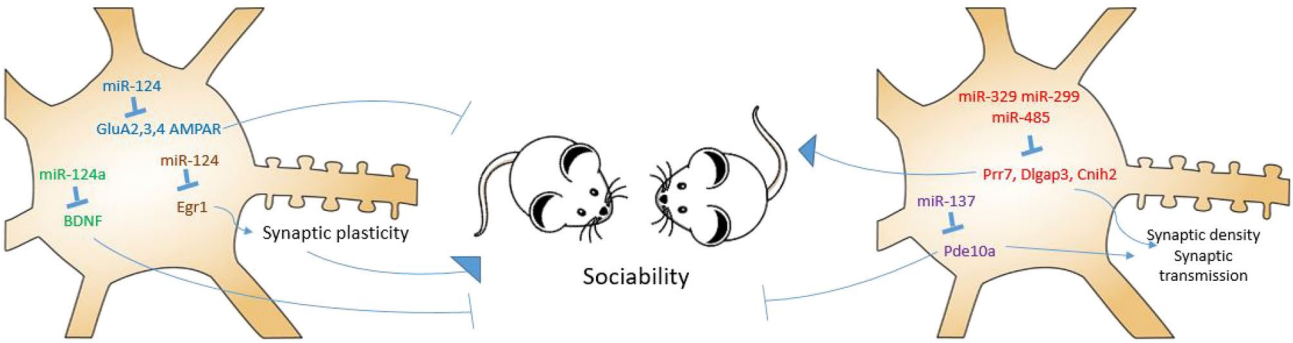

Fig. 2 Schematic linking miRNAs and their targets involved in sociability identified through functional studies in mouse models

levels in saliva of ASD patients identified miR-146 as one of the reliable diagnostic markers [61]. Apart from ASD, increased miR-146a levels were also observed in temporal lobe epilepsy [62] and frontal cortical dysplasia [63], and decreased levels of miR-146b was reported in the hippocampus of mice harbouring BDNF Val66Met SNP associated with human depressive- and anxiety-like traits [64]. These results point towards a possible convergence of molecular mechanisms in these disorders and ASD. One such mechanism is altered inflammatory responses, as evidenced by miR-146a KO mouse models displaying severe autoimmune diseases, enlarged spleen and premature death [65, 66]. Taken together, multiple lines of evidence point to an important role of miR-146a dysregulation in the aetiology of several neuropsychiatric conditions, including ASD. Given the widespread expression of miR-146a in multiple cell types in the brain, including neurons, astrocytes, immune and endothelial cells, tissue-specific miR-146a knockout models are likely required to get more insight into miR-146a regulated cellular pathways that are relevant for the control of ASD-related behaviours.

miR-132

Another miRNA implicated in human expression studies is miR-132. Studies conducted with human lymphoblastoid

cell lines show a significant dysregulation of miR-132 related to autism [52, 53]. However, whereas one study reported miR-132 upregulation in ASD patients compared to healthy controls [53], another study that used co-twins/siblings for the analysis reported the opposite trend [52]. A role for miR-132 in the control of neuronal development and function is documented by both cell culture and in vivo animal studies. By regulating the expression of Foxp2, a transcription factor associated with language disorders [67], miR-132 together with miR-9 regulates neurite outgrowth and radial neuronal migration in the mouse cortex [68]. Moreover, keeping miR-132 expression within a physiological range was found to be important for maintaining synaptic plasticity in the mouse visual cortex [69]. In transgenic mice, overexpression of miR-132 in mouse forebrain pyramidal neurons resulted in impaired cognitive behaviour as assessed by deficits in the novel object recognition tasks [70]. One of the validated targets of miR-132 is methyl CpG-binding protein 2 (MeCP2), a transcriptional repressor that is implicated in a wide spectrum of neurological disorders, including ASD, Rett syndrome and ID [71]. In addition to its role in transcriptional repression, phosphorylated MeCP2 was also shown to directly bind to DGCR8 protein. Thereby, it sequesters DGCR8 away from the microprocessor enzyme Drosha, impairing the formation of mature microRNAs such as miR-134, a critical member of the miR379-410 cluster implicated in the regulation of social behaviour [72]. Interestingly, miR-132 and miR-137 mediate reciprocal regulation between MeCP2 and phosphatase and tensin homolog (PTEN), another autism-related gene [73], highlighting the significance of miRNA mediated mechanisms in maintaining balance in expression of key ASD genes. In addition, MeCP2 is also regulated by miR-483 [74]. In summary, while an important role of miR-132 in synaptic plasticity and cognition is well established, the links to ASD are mostly correlative. More insight into the role of miR-132 in ASD can be expected from the characterization of ASD-related behaviours, e.g. social and repetitive behaviour and communication, in miR-132 loss-of-function mouse models.

Role of miRNAs in autism spectrum disorder—insights from genetic association studies

Studies in the last decade have recognized copy number variants (CNVs) as one of the important factors contributing to ASD through analysis of protein-coding genes [75, 76]. However, deleted and duplicated CNV loci hosting miRNA genes may similarly lead to dosage imbalance of the miRNA target genes, thereby resulting in functional implications related to ASD. So far, two studies have attempted to link miRNA genes and autism-associated CNV loci. By analyzing 378 CNVs in Autism Database (AutDB) that are consistently reported to be associated with ASD, 42 CNV loci were identified that harbour a total of 72 miRNAs [43]. Interestingly, expression analysis showed that some of these miRNAs—miR-484, miR-598, miR-7, miR-195 and miR-211—were also deregulated in human post-mortem brain and lymphoblastoid cell lines derived from ASD patients [45, 51, 52]. Using a computational method, another study tested over-representation of miRNA genes in ASD-associated de novo CNVs [77]. Of the total 178 de novo CNVs from ASD patients, 64 overlapped with at least one miRNA gene. Interestingly, 8 miRNA genes were reported in both of these studies—MIR429, MIR200a, MIR200b, MIR149; MIR85, MIR1306, MIR1286 and MIR649. However, very little is currently known regarding the function of these miRNA candidates in the context of the nervous system, and additional experimental studies are clearly warranted.

Single nucleotide polymorphisms (SNPs) within miRNA genes have been reported to be rare. Only an estimated 10% of human pre-miRNAs and < 1% of miRNAs (in the seed region) have SNPs [78, 79]. Variants in pre-miRNA genes that locate outside the seed region can also significantly alter miRNA expression, maturation and interaction with its target mRNAs [80, 81]. A recent case–control association study was performed by genotyping 350 common SNPs targeting 163 miRNA genes and clusters in ASD patients and controls [40]. This study reported five SNPs in MIR219-1, cluster MIR133b/MIR206, cluster MIR106b/MIR93/MIR25 and the MIR17/MIR18a/MIR19a/MIR20a/MIR19b-1/MIR92a-1 cluster. Through whole-exome sequencing, the same group studied the presence of rare variants in 701 pre-miRNA genes in 101 individuals from 30 ASD families. Although no variants were reported in the seed region, nine changes outside the seed region in the mature miRNA were identified. These changes were predicted to affect the hairpin stability and hence functionality of the miRNAs. Interestingly, three of these variants—MIR1182, MIR1914 and MIR589—are mapped to ASD-associated CNVs [40]. These studies indicate that changes in miRNA levels resulting from CNVs and SNPs may contribute to the development of ASD and set the basis for mechanistic studies in human cellular models, such as induced pluripotent stem cell (IPSC)-derived neurons.

In addition to genetic risk factors, environmental factors are also surfacing to be important players in the aetiology of ASD. Although no single factor accounts for the increased prevalence of ASD, chemicals, infectious agents, dietary factors and physical/psychological stressors are known as environmental risk factors [82]. Polyphenols, an abundant class of dietary component present in many vegetables and fruits, has been found to regulate the expression of several miRNAs implicated in pathologies such as inflammation, cancer, neurodegeneration and aging [83]. Interestingly, several miRNAs discussed here in the context of social

behaviour and ASD were shown to be regulated by polyphenols. For example, three members of the miR379-410 cluster, miR-377, miR-376a and miR-654 are regulated by 13C, DIM and Isoflavone [84]. Another miRNA that prominently appeared in several profiling studies, miR-146 was shown to be regulated by 13C, DIM, Isoflavone and Resveratrol [84]. Another study demonstrated that exposure to pesticides altered miRNAs implicated in neurological functions such as neurotransmission (miR-517b, miR-133b, miR-597) [85]. Further functional studies are needed to disentangle these rather complex gene-environment interactions, with a focus on underlying mechanism and critical time period of action of these factors towards developing social deficits.

Role of miRNA in anxiety

Anxiety is an evolutionary trait and an organism's response to cope with adverse environmental stimuli. It is associated with emotional, cognitive functions that are orchestrated by multiple and highly plastic neural centres and neurotransmitter pathways [86]. Anxiety disorders comprise of acute stress disorder, obsessive–compulsive disorder (OCD), post-traumatic stress disorder (PTSD), panic disorder (PD) and phobias. Besides the implication of amygdala in the manifestation of anxiety and stress, studies reveal that the parahippocampal gyrus, cingulate cortex and frontal cortex display heightened activity in response to anxiety-inducing stimuli [87, 88]. These findings collectively suggest an important role of the forebrain in the aetiology of anxiety disorders, with excessive excitatory neurotransmission being one of the physiological hallmarks [89].

Role of miRNAs in anxiety-like behaviour—evidences from functional studies

miR-34 family

In 2011, the Chen lab provided first functional evidence for an important role of miRNA-dependent gene regulation in anxiety-related behaviours. Lentiviral-mediated local ablation of the miRNA processing enzyme Dicer in the central amygdala (CeA) of adult mice resulted in increased anxiety-like behaviour with no significant effect on neuronal survival and morphology [90]. This highlights the important contribution of miRNA machinery to the functional regulation of the central stress response, deregulation of which is linked to the aetiology of anxiety and mood disorders. In response to both acute and chronic stress, miR-34c was found to be upregulated. Moreover, lentiviral mediated overexpression of miR-34c in the adult CeA conferred anxiolytic behaviour after stress induction, demonstrating that stress-mediated upregulation of miR-34c is functionally relevant to counteract abnormal behaviour. Through an evolutionarily conserved binding site in the 3′-UTR, miR-34c targets the stress-related corticotropin releasing factor receptor type 1 (CRFR1) mRNA, and reduces responsiveness of neuronal cells to CRF [90].

Surprisingly, genetic deletion of all three members of the miR-34 family in mice resulted in resilience to acute stress-induced anxiety and facilitation in fear extinction [91], somehow at odds with the results from Haramati et al. Using intracerebral in vivo microdialysis, the study found no significant increase in aminergic GABA release in the prefrontal cortex or amygdala and no stress-induced amygdalar dendritic remodelling. However, differential expression of GRM7, 5-HT2C, and CRFR1 mRNA expression was noted in the mPFC and basolateral amygdala (BLA) of the KO mice [91].

In another recent study, overexpression of miR-34b using miRNA mimics in the paraventricular nucleus (PVN) of rats resulted in decreased hyperactivity of the HPA axis and anxiety-like behaviour, possibly through its interaction with corticotropin-releasing hormone receptor 1 (CRHR1) [92].

miR-132/212

A recent study demonstrates that exposure to stress alters the expression of miR-132 and miR-212, two miRNAs that are expressed from the same non-coding transcript. Following acute stress (5 h), both miRNAs are upregulated more than two-fold in the mouse hippocampus and amygdala, whereas following chronic stress (15 days) the upregulation was observed only in the amygdala [93]. Interestingly, miR-132 overexpression and miR-132/212 conditional knockout mouse models both displayed increased basal anxiety-like behaviour, suggesting that keeping miR-132/212 levels in a physiological window is critical to suppress anxiety. At the molecular level, two miR-132 target genes Sirt1 and PTEN were differentially regulated in the hippocampus and amygdala of the transgenic mice. This indicates that the cellular level of miR-132 and miR-212, through the regulation of these anxiety-relevant target genes, is crucial in modulating stress responsivity and anxiety [93].

miR-101a-3p

In rats selectively bred for differences in emotionality and stress reactivity, the levels of miR-101a-3p were found to correlate with the traits observed—high novelty responding rats with low anxiety had lower miR-101a-3p levels in the amygdala, whereas the low novelty responding rats with high anxiety displayed the opposite trend [94]. Subsequently,

viral-mediated overexpression of miR-101a-3p in the amygdala of high novelty responding rats resulted in increased anxiety-like behaviour as assessed through open field test and elevated plus maze (EPM). This manipulation also resulted in reduced levels of the histone methyltransferase Ezh2, which mediates gene silencing via tri-methylation of histone 3 at lysine 27 (H3K27me3). Knockdown of Ezh2 resulted in behavioural phenotypes which were similar to those observed upon miR-101a-3p overexpression, albeit to a lesser extent [94], suggesting that miR-101 and Ezh2 might indeed be in the same pathway.

miR-155

miR-155 is a microRNA that is predominantly studied in the immune system, although it recently emerged also as stress-regulated miRNA in the nervous system. MiR-155 KO mice displayed reduced anxiety-like behaviour by spending more time in open areas in the open field and elevated plus maze [95]. In addition, the KO mice displayed reduced float duration and increased latency to float in the forced swim test, suggesting decreased depression-like behaviour. Since there were no deficits found in learning and memory or social preference/novelty in these mice, miR-155 seems to regulate specifically anxiety-like affective behaviours [95]. In line with the reported function of miR-155 in immune cells, analysis of hippocampi from miR-155 KO mice showed reduced expression of inflammatory cytokines, such as IL-6 and TNF-a, compared to control animals.

miR-17-92 cluster

We have already discussed this miRNA cluster in the context of ASD, but dysregulation of miR-17-92 expression has also been implicated in anxiety and mood disturbances. Deletion of the miR-17-92 cluster in adult neural progenitors results in decreased neurogenesis in the dentate gyrus while its overexpression results in the opposite outcome [38]. Genes in the glucocorticoid pathway were identified as direct targets of the cluster, particularly serum- and glucocorticoid-inducible protein kinase-1 (Sgk1), providing a link to stress-related pathways. Behaviourally, miR-17-92 KO mice display anxiety- and depression-like behaviours, whereas miR-17–92 overexpression leads to opposite phenotypes. In addition, ectopic miR-17-92 expression rescues proliferation defects induced by corticosterone in hippocampal neural progenitors [38]. Together, this is one of the few studies where a family of miRNAs was shown to bi-directionally control anxiety and depression-like behaviours. Since miR-17-92 is also involved in the regulation of ASD-related behaviours, it might be a promising target for therapeutic intervention in neurodevelopmental and psychiatric conditions.

miR-135a

In a transgenic mouse model, overexpression of miR-135a specifically in serotonergic neurons in the Raphe nucleus leads to reduced anxiety- and depression-like behaviours after social defeat as measured by dark–light transfer and elevated plus maze tests [96]. On the other hand, miR-135a knockdown resulted in increased anxiety-like behaviour and decreased response to antidepressants. Subsequently, miR-135a has been found to interact with serotonin transporter and serotonin receptor-1a, thereby modulating depression- and anxiety-like behaviours. Interestingly, miR-135a levels were significantly reduced in the blood of depressed patients compared to healthy controls, whereas miR-135a levels were increased upon antidepressant treatment. Collectively, these results indicate that miR-135a contributes to stress-resilience and emerges as a potential biomarker for depression diagnosis and treatment response [96].

Another recent study also investigated the role of miR-135a in anxiety-like behaviour but focussing on its function in the amygdala. Knockdown of miR-135a in the amygdala leads to increased anxiety-like behaviour which is paralleled by an increase in spontaneous excitatory postsynaptic currents in amygdala acute brain slices [97]. In addition, through in vivo miRNA overexpression analysis, regulators of synaptic vesicle fusion (complexin-1 and complexin-2) were identified as direct targets of miR-135a. Interestingly, upon exposure to acute stress, downregulation of miR-135a and concomitant upregulation of complexin-1 and complexin-2 were observed in the mouse amygdala, unravelling a novel mechanism of miRNA regulation of anxiety-like behaviours in the amygdala through modulation of presynaptic glutamatergic neurotransmission [97]. Since miR-137 was shown to control a similar set of presynaptic targets related to schizophrenic behaviour [34], it would be interesting to determine the interaction of these two miRNAs in the control of synaptic transmission in the healthy and diseased brain.

miR379-410 cluster

The large miR-379-410 cluster of placental mammal-specific paternally imprinted miRNAs, which had been previously linked to the regulation of energy homeostasis, was also implicated in anxiety-like behaviour. In one study [98], the deletion of the miR379-410 cluster in mice led to increased anxiety, however with no changes in locomotion, spontaneous exploration, learning, spatial memory and sociability. Increased levels of anxiety-like behaviours were later on confirmed in an independent miR379-410 KO model [22], however in this case accompanied by hypersociability. This rare combination of anxiety and hypersociability phenotype is reminiscent of Williams and Angelman Syndrome. Future

Table 2 List of miRNAs implicated in anxiety-like behaviour through functional studies in animal models

miRNA	Animal model	Behavioural phenotype	Molecular mechanism	References
miR-34 family/miR-34c	Lentiviral overexpression of miR-34c in central amygdala	Anxiolytic behaviour after stressful challenge	Targets the stress-related corticotropin releasing factor receptor type 1 (CRFR1) mRNA, and reduces responsiveness of neuronal cells to CRF	[90]
miR-34 family	Deletion of all three members in mice	Resilience to acute stress-induced anxiety and facilitation in fear extinction	Differential expression of GRM7, 5-HT2C, and CRFR1 mRNA expression in mPFC and BLA	[91]
miR-34 family/miR-34b	Overexpression of miR-34b using miRNA agomir in paraventricular nucleus of rat	Decreased hyperactivity of the HPA axis and anxiety-like behaviour	Interaction with corticotropin-releasing hormone receptor 1 (CRHR1)	[92]
miR-132/miR-212	Overexpression and conditional knockout in mice	Increased basal anxiety-like behaviour	Sirt1 and Pten were differentially expressed in hippocampus and amygdala	[93]
miR-101a-3p	Overexpression of miR-101a-3p in amygdala of high novelty responding rats	Increased anxiety-like behaviour	Reduced levels of histone methyltransferase Ezh2	[94]
miR-155	Knockout of miR-155 in mice	Reduced anxiety-like behaviour, decreased depression-like behaviour	Reduced inflammation-specific genes IL-6, TNF-a in hippocampus	[95]
miR-17-92 cluster	Knockout of the cluster	Increased anxiety- and depression-like behaviour	Targets the glucocorticoid pathway, particularly Sgk1	[38]
miR-135a	Knockdown of miR-135a in mouse amygdala	Increased anxiety-like behaviour	Increase in spontaneous excitatory postsynaptic currents Increased levels of regulators of synaptic vesicle fusion complexin-1 and complexin-2	[97]
miR-135a	Overexpression of miR-135a in mouse raphe nucleus	Decreased anxiety- and depression-like behaviour upon social defeat	Strong interaction with serotonin transporter and serotonin receptor 1a	[96]
miR379-410 cluster	Constitutive germline deletion of the cluster in mice	Increased anxiety-like behaviour	Increased excitatory synapse-specific gene expression and decreased inhibitory synapse-specific gene expression in the hippocampus	[22, 98]

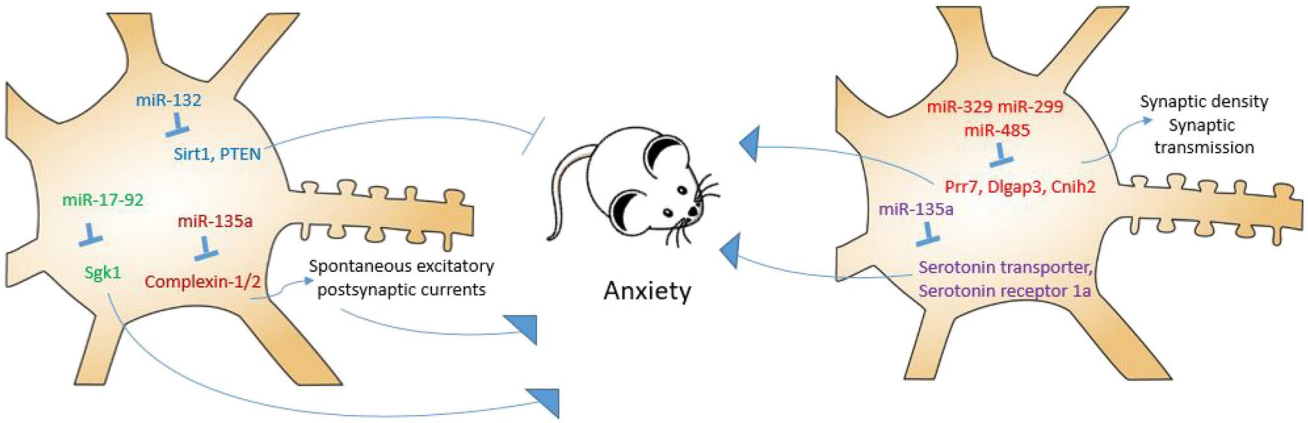

Fig. 3 Schematic linking miRNAs and their targets involved in anxiety identified through functional studies in mouse models

studies are needed to evaluate if miR379-410 knockout mice could indeed serve as an animal model for one of these rare neurodevelopmental conditions. Table 2 provides a summary of miRNAs implicated in anxiety-like behaviour through functional studies in animal models. Figure 1 displays the corresponding brain regions involved and Fig. 3 provides an overview of the underlying molecular mechanisms.

Role of miRNAs in anxiety disorder—insights from profiling and genetic association studies

One of the first studies that provided correlative links between miRNAs and anxiety performed differential miRNA expression profiling in the hippocampus of four inbred mouse strains. The expression of two miRNAs (miR-34c, miR-323) correlated with anxiety levels based on results from an elevated plus maze (EPM) test, whereas the expression of four miRNAs each correlated either with explorative behaviour (miR-34a, miR-323, miR-378, miR-451) or learning and memory (miR-34c, miR-323, miR-378, and miR-451) [99]. Similarly, acute restraint stress was also shown to upregulate expression levels of various miRNAs (let-7a, miR-9 and miR-26-a/b) in the frontal cortex but not hippocampus of CD1 mice, whereas only minor changes were observed after repeated restraint stress. Based on these results, the authors of the study concluded that acute stress elicited rapid, but rather transient changes in miRNA levels [100]. In contrast, an earlier study performed in rats indicated that repeated restraint stress leads to reduced levels of glucocorticoid receptor (GR) in the PVN in response to chronically increased levels of miR-18a [101]. In addition to the forebrain, chronic stress also results in altered levels of certain miRNAs (increased miR-186, miR-381 and decreased miR-709) in the cerebellum [102]. These studies indicate that region-dependent changes in miRNAs in response to both acute and chronic stress might modulate the susceptibility to stress-related disorders.

In a model of visceral hypersensitivity and anxiety—chronic water avoidance stress—39 miRNAs were found to be differentially regulated by stress in the spinal cord [103]. Particularly, significant upregulation of miR-17-5p was found in the stressed rats compared to controls, which led to a subsequent change in the expression of targets which function in both inflammatory (IL-6, JAK/STAT, TNF) and metabolic (PI3K/AKT) signalling pathways. In addition, miR-17-5p was demonstrated to have a modulatory role in visceral sensitivity in vivo [103]. Depressive-like phenotype upon chronic stress exposure was also shown to result in increased levels of miR-326 in the nucleus accumbens, while levels of the same miRNA decreased in the striatum [104].

MiRNA expression changes upon stress were also investigated in the amygdala, a structure strongly implicated in fear processing. Here, the exposure of rats to acute stress resulted in the upregulation of miR-134 and miR-183 [105], whereas, upon chronic stress exposure, the levels of miR-134 were shown to be reduced. Mechanistically, the altered expression of these miRNAs resulted in changes in alternative splicing of acetylcholinesterase (AChE), thereby affecting cholinergic neurotransmission under stress conditions [105]. Another study established that miR-186 regulates both AChE and also major peripheral cholinesterase (BChE) in mice exposed to predator stress, such that the levels of these targets were elevated 1 week post-exposure [106].

Maternal separation as a model of early-life adversity and stress results in increased levels of pre-miR-132, -124-1, -9-1, -9-3, -212, and -29a as well as the mature miR-132, -124, -9, and -29a in the medial prefrontal cortex (mPFC) of rats [107]. The function of miR-9 in the stress response remains controversial since miR-9 downregulation was found to be linked to increased susceptibility to anxiety and depression in the context of early-life stress by targeting a dopamine receptor subunit (DRD2) in a more recent study [104]. Intriguingly, altered miRNA expression caused by traumatic early-life stress appear to be transmitted across generations and might even play a causal role in the heritability of adverse behaviours. In a mouse model of maternal separation and unexpected stress (MSUS), a large set of miRNAs (miR-375-3p, miR-375-5p, miR-200b-3p, miR-672-5p, and miR-466-5p) were upregulated in the sperm of MSUS males [107]. Interestingly, stress-dependent changes in the expression levels of these miRNAs (except miR-200b-3p) were comparable between F1 sperm and F2 hippocampus, suggesting the existence of mechanisms that reinstate miRNA expression in the next generations. In addition, injection of sperm RNAs from stressed males into fertilized wild-type oocytes resulted in the same behavioural and metabolic changes in the offspring [107]. Although these findings are consistent with a causal role of miRNAs in epigenetic inheritance of adverse life events, the contribution of other RNA species, both coding (mRNAs) and non-coding (e.g. lncRNAs, piRNAs, circRNAs, etc.) to this phenomenon cannot be ruled out. In fact, a recent study from the same group established that long RNAs from sperm significantly contribute to the epigenetic inheritance of adverse life events [108].

On the other side of the spectrum, a potentially positive effect of environmental enrichment on anxiety has been recently explored in rodents [109]. Interestingly, miR-124 was found to be upregulated in response to environmental enrichment, ultimately resulting in improved cognition and neurogenesis in the rat dentate gyrus [110]. In agreement with a positive regulatory role of miR-124 in the context of anxiety, it was shown in another study that lentiviral mediated silencing of miR-124a increased neonatal isolation-induced

anxiety-like behaviour based on results from the open field and elevated plus-maze test [28]. Interestingly, this manipulation also resulted in increased interaction in social behaviour test, suggesting a dual role for miR-124 in circuits mediating anxiety-related and social behaviour.

GWAS and case–control association studies have uncovered many genetic variants associated with anxiety, each of which however does not contribute a high percentage to the heritability of anxiety disorders. This reflects the complex interplay between genetic, epigenetic and environmental factors that contribute to the disorder. Variations in miRNAs that regulate expression of RGS2, a gene previously associated with anxiety-related phenotypes and PD [111, 112], were investigated using bioinformatics and reporter assays [113]. Four algorithms predicted several miRNAs that were able to regulate RGS2 expression and subsequent disruption of the seed sequences of these miRNAs resulted in elevated expression of reporter genes. Hsa-miR-4717-5p exhibited the most pronounced effect on RGS2 expression and also regulated two other anxiety candidate genes (IKBKE and CNR1). Furthermore, two SNPs (rs150925, rs161427) within a 1000 bp upstream of the host gene of hsa-miR-4717-5p, MIR4717, showed a trend for association with PD. It was therefore proposed that hsa-miR-4717-5p regulates human RGS2 and thereby contributes to the genetic risk toward anxiety-related traits, pointing towards novel miRNA-regulated gene networks involved in anxiety disorders [113].

In addition to investigating variants within miRNA genes, investigations into the expression levels of miRNAs could provide first insights about possible links between miRNA dysregulation, anxiety and other stress-related disorders. Case–control studies analysing SNPs in 325 human miRNA regions in a cohort of panic disorder patients identified polymorphisms in the miR-22, miR-138-2, miR-148a and miR-488 genes [114]. These miRNAs were found to regulate several candidate genes such as BDNF, GABRA6, CCKBR, POMC, HTR2C, MAOA and RGS2, which are associated with PD and anxiety disorders. Another recent study focusing on depression showed that expression levels of miR-144-5p in plasma were inversely associated with depression scores and that levels of miR-144-5p were significantly lower in depressed patients than in healthy controls. Furthermore, following treatment, plasma levels of miR-144-5p significantly increased in depression/anxiety patients and were significantly higher than levels measured at baseline [115].

Conclusions

The role of miRNAs in post-transcriptional regulation of gene targets in different cellular context is now well established—from robust regulation of entire pathways during development to the local fine-tuning of specific targets in neuronal processes (for a detailed review see [116]). Strikingly, studies over the last decade are beginning to uncover how this class of small non-coding RNA is influencing animal behaviour. Here in this review, we have provided an overview of the most promising miRNAs linked to changes in sociability and anxiety through functional studies in animal models. In addition, we have highlighted the key overlapping findings originating from association and expression profiling studies in human samples.

Animal models provide a valuable approach to link changes in miRNA biogenesis and molecular action to pathological and behavioural changes relevant for neuropsychiatric disorders [117]. Targeting either genes encoding for microRNAs themselves, microRNA biogenesis factors or microRNA target genes, animal models with knock-out, knock-down, or overexpression of specific gene can be performed in a spatial and/or temporal manner followed by a detailed analysis of the resulting molecular and behavioural phenotypes. In addition, molecular tools such as miRNA mimics, anti-miRNAs or miRNA sponges can be employed to manipulate miRNA-related gene expression changes in an even more acute and regionally defined manner [117]. Consistent with recent observations that prevalent neuropsychiatric disorders have large genetic overlaps, specific miRNAs have been repeatedly implicated in different mental diseases based on these functional studies. For instance, miR-137, miR-124 and the miR379-410 cluster, besides regulating social behaviour, have also been implicated in schizophrenia, frontotemporal dementia and anxiety, respectively.

At the molecular and cellular level, several of these miRNAs target components of the NMDA, AMPA receptors and second messenger signalling cascades, suggesting that dysregulation of these systems might be a common denominator of mental illness. Nevertheless, there are also likely specific miRNA-dependent regulations in anxiety and social dysfunction. For example, the social behaviour disorders spectrum is often characterized by the dysregulation of synaptic pruning, dendritic growth and the balance between excitatory and inhibitory synaptic transmission, specifically in the hippocampus and forebrain. On the other hand, concerning the anxiety-related behaviour spectrum, several miRNAs regulate corticotropin-releasing factor signalling, excitatory postsynaptic transmission and synaptic vesicle fusion, among others. It will be interesting for future studies to investigate the expression levels of these miRNAs in a more brain-region (prefrontal cortex, hippocampus, amygdala, striatum) and cell-type (pyramidal neurons, inhibitory neurons, glia and microglia) specific manner, using techniques such as single-cell sequencing and single-molecule fluorescence in situ hybridization (smFISH). This, when complemented with advanced manipulation techniques such as CRISPR/Cas9,

AAV/Lentiviral approaches, will yield greater insights into the cell-type-specific function of these miRNAs and underlying molecular mechanisms.

While the use of animal models yields valuable insights into the disease mechanism, investigating the relevance of such miRNA candidates to human disease aetiology is crucial. Though there is only limited number of miRNA expression profiling studies done in humans displaying anxiety and stress, numerous expression profiling studies have been done in human samples of autism spectrum disorders (ASD) ranging from blood serum, peripheral blood mononuclear cells, lymphoblastoid cell lines to post mortem brain samples as discussed here. However, the number of miRNAs that overlap among these studies are few and the direction of expression changes is not always consistent between the studies. This could arise from a range of factors such as tissue source, age, sensitivity of miRNA assay and statistical analysis criteria among others. Despite such differences, miR-146a appears to be strongly linked to ASD pathology, a miRNA which is also implicated in neurodevelopment, synaptic transmission and regulation of ASD-specific genes through both animal studies and cell culture experiments. Since the knockout of miR-146a leads to premature death in mice [65, 66], it is important to develop brain-specific conditional knockout model to study the gene network affected by miR-146a dysregulation.

In conclusion, to be able to fully understand the significance of miRNA in the onset and progression of social and anxiety disorders, future studies should be targeted towards expression profiling in larger patient cohorts and greater coverage of miRNAs and complemented by functional studies in animal models. Significant advancements in the field of stem cells also offer promising methods to develop human induced pluripotent stem cell-based models for ASD and anxiety-related disorders and to study the role of human-specific miRNAs at the functional level. This will be imperative to advance the use of miRNAs in the diagnosis and therapy of complex neuropsychiatric conditions.

Acknowledgements We apologize to colleagues whose work we were not able to discuss due to space limitations. Work in the Schratt laboratory is in part funded by grants from the Swiss National Science Foundation (SNSF; 310030E_179651, 32NE30_189486), Deutsche Forschungsgemeinschaft (DFG; FI 2157/2-1, DI 1501/5-2) and ETH Zurich Marie Curie COFUND postdoc fellowship to R. Narayanan.

References

1. Lee RC, Feinbaum RL, Ambros V (1993) The *C. elegans* heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. Cell 75(5):843–854. https://doi.org/10.1016/0092-8674(93)90529-y
2. Wightman B, Ha I, Ruvkun G (1993) Posttranscriptional regulation of the heterochronic gene lin-14 by lin-4 mediates temporal pattern formation in *C. elegans*. Cell 75(5):855–862. https://doi.org/10.1016/0092-8674(93),90530-4
3. Reinhart BJ, Slack FJ, Basson M, Pasquinelli AE, Bettinger JC, Rougvie AE, Horvitz HR, Ruvkun G (2000) The 21-nucleotide let-7 RNA regulates developmental timing in *Caenorhabditis elegans*. Nature 403(6772):901–906. https://doi.org/10.1038/35002607
4. Ha M, Kim VN (2014) Regulation of microRNA biogenesis. Nat Rev Mol Cell Biol 15(8):509–524. https://doi.org/10.1038/nrm3838
5. Krol J, Loedige I, Filipowicz W (2010) The widespread regulation of microRNA biogenesis, function and decay. Nat Rev Genet 11(9):597–610. https://doi.org/10.1038/nrg2843
6. Winter J, Jung S, Keller S, Gregory RI, Diederichs S (2009) Many roads to maturity: microRNA biogenesis pathways and their regulation. Nat Cell Biol 11(3):228–234. https://doi.org/10.1038/ncb0309-228
7. Meister G (2013) Argonaute proteins: functional insights and emerging roles. Nat Rev Genet 14(7):447–459. https://doi.org/10.1038/nrg3462
8. Filipowicz W, Bhattacharyya SN, Sonenberg N (2008) Mechanisms of post-transcriptional regulation by microRNAs: are the answers in sight? Nat Rev Genet 9(2):102–114. https://doi.org/10.1038/nrg2290
9. Hammond SM (2015) An overview of microRNAs. Adv Drug Deliv Rev 87:3–14. https://doi.org/10.1016/j.addr.2015.05.001
10. Kim VN, Han J, Siomi MC (2009) Biogenesis of small RNAs in animals. Nat Rev Mol Cell Biol 10(2):126–139. https://doi.org/10.1038/nrm2632
11. Cheloufi S, Dos Santos CO, Chong MM, Hannon GJ (2010) A dicer-independent miRNA biogenesis pathway that requires Ago catalysis. Nature 465(7298):584–589. https://doi.org/10.1038/nature09092
12. Abdelfattah AM, Park C, Choi MY (2014) Update on non-canonical microRNAs. Biomol Concepts 5(4):275–287. https://doi.org/10.1515/bmc-2014-0012
13. Baskerville S, Bartel DP (2005) Microarray profiling of microRNAs reveals frequent coexpression with neighboring miRNAs and host genes. RNA 11(3):241–247. https://doi.org/10.1261/rna.7240905
14. Lau NC, Lim LP, Weinstein EG, Bartel DP (2001) An abundant class of tiny RNAs with probable regulatory roles in *Caenorhabditis elegans*. Science 294(5543):858–862. https://doi.org/10.1126/science.1065062
15. Lagos-Quintana M, Rauhut R, Lendeckel W, Tuschl T (2001) Identification of novel genes coding for small expressed RNAs. Science 294(5543):853–858. https://doi.org/10.1126/science.1064921
16. Lee RC, Ambros V (2001) An extensive class of small RNAs in *Caenorhabditis elegans*. Science 294(5543):862–864. https://doi.org/10.1126/science.1065329
17. Bartel DP (2009) MicroRNAs: target recognition and regulatory functions. Cell 136(2):215–233. https://doi.org/10.1016/j.cell.2009.01.002
18. Huntzinger E, Izaurralde E (2011) Gene silencing by microRNAs: contributions of translational repression and mRNA decay. Nat Rev Genet 12(2):99–110. https://doi.org/10.1038/nrg2936
19. Schratt GM, Tuebing F, Nigh EA, Kane CG, Sabatini ME, Kiebler M, Greenberg ME (2006) A brain-specific microRNA regulates dendritic spine development. Nature 439(7074):283–289. https://doi.org/10.1038/nature04367
20. Siegel G, Obernosterer G, Fiore R, Oehmen M, Bicker S, Christensen M, Khudayberdiev S, Leuschner PF, Busch CJ, Kane C, Hubel K, Dekker F, Hedberg C, Rengarajan B, Drepper C,

Waldmann H, Kauppinen S, Greenberg ME, Draguhn A, Rehmsmeier M, Martinez J, Schratt GM (2009) A functional screen implicates microRNA-138-dependent regulation of the depalmitoylation enzyme APT1 in dendritic spine morphogenesis. Nat Cell Biol 11(6):705–716. https://doi.org/10.1038/ncb1876

21. Buiting K, Williams C, Horsthemke B (2016) Angelman syndrome—insights into a rare neurogenetic disorder. Nat Rev Neurol 12(10):584–593. https://doi.org/10.1038/nrneurol.2016.133
22. Lackinger M, Sungur AO, Daswani R, Soutschek M, Bicker S, Stemmler L, Wust T, Fiore R, Dieterich C, Schwarting RK, Wohr M, Schratt G (2019) A placental mammal-specific microRNA cluster acts as a natural brake for sociability in mice. EMBO Rep. https://doi.org/10.15252/embr.201846429
23. Yin CL, Chen HI, Li LH, Chien YL, Liao HM, Chou MC, Chou WJ, Tsai WC, Chiu YN, Wu YY, Lo CZ, Wu JY, Chen YT, Gau SS (2016) Genome-wide analysis of copy number variations identifies PARK2 as a candidate gene for autism spectrum disorder. Mol Autism 7:23. https://doi.org/10.1186/s13229-016-0087-7
24. Rascovsky K, Hodges JR, Knopman D, Mendez MF, Kramer JH, Neuhaus J, van Swieten JC, Seelaar H, Dopper EGP, Onyike CU, Hillis AE, Josephs KA, Boeve BF, Kertesz A, Seeley WW, Rankin KP, Johnson JK, Gorno-Tempini ML, Rosen H, Prioleau-Latham CE, Lee A, Kipps CM, Lillo P, Piguet O, Rohrer JD, Rossor MN, Warren JD, Fox NC, Galasko D, Salmon DP, Black SE, Mesulam M, Weintraub S, Dickerson BC, Diehl-Schmid J, Pasquier F, Deramecourt V, Lebert F, Pijnenburg Y, Chow TW, Manes F, Grafman J, Cappa SF, Freedman M, Grossman M, Miller BL (2011) Sensitivity of revised diagnostic criteria for the behavioural variant of frontotemporal dementia. Brain 134:2456–2477. https://doi.org/10.1093/brain/awr179
25. Gao FB (2010) Context-dependent functions of specific microRNAs in neuronal development. Neural Dev. https://doi.org/10.1186/1749-8104-5-25
26. Gascon E, Lynch K, Ruan HY, Almeida S, Verheyden JM, Seeley WW, Dickson DW, Petrucelli L, Sun DQ, Jiao J, Zhou HR, Jakovcevski M, Akbarian S, Yao WD, Gao FB (2014) Alterations in microRNA-124 and AMPA receptors contribute to social behavioral deficits in frontotemp oral dementia. Nat Med 20(12):1444–1451. https://doi.org/10.1038/nm.3717
27. Yang Y, Shu X, Liu D, Shang Y, Wu Y, Pei L, Xu X, Tian Q, Zhang J, Qian K, Wang YX, Petralia RS, Tu W, Zhu LQ, Wang JZ, Lu Y (2012) EPAC null mutation impairs learning and social interactions via aberrant regulation of miR-124 and Zif268 translation. Neuron 73(4):774–788. https://doi.org/10.1016/j.neuron.2012.02.003
28. Bahi A (2017) Hippocampal BDNF overexpression or microR124a silencing reduces anxiety- and autism-like behaviors in rats. Behav Brain Res 326:281–290. https://doi.org/10.1016/j.bbr.2017.03.010
29. Cheng Y, Wang ZM, Tan WQ, Wang XN, Li YJ, Bai B, Li YX, Zhang SF, Yan HL, Chen ZL, Liu CM, Mi TW, Xia ST, Zhou ZK, Liu A, Tang GB, Liu C, Dai ZJ, Wang YY, Wang H, Wang XS, Kang YH, Lin L, Chen ZP, Xie NN, Sun QM, Xie W, Peng JM, Chen DH, Teng ZQ, Jin P (2018) Partial loss of psychiatric risk gene Mir137 in mice causes repetitive behavior and impairs sociability and learning via increased Pde10a. Nat Neurosci 21(12):1689. https://doi.org/10.1038/s41593-018-0261-7
30. Olde Loohuis NF, Ba W, Stoerchel PH, Kos A, Jager A, Schratt G, Martens GJ, van Bokhoven H, Nadif Kasri N, Aschrafi A (2015) MicroRNA-137 controls AMPA-receptor-mediated transmission and mGluR-dependent LTD. Cell Rep 11(12):1876–1884. https://doi.org/10.1016/j.celrep.2015.05.040
31. Carter MT, Nikkel SM, Fernandez BA, Marshall CR, Noor A, Lionel AC, Prasad A, Pinto D, Joseph-George AM, Noakes C, Fairbrother-Davies C, Roberts W, Vincent J, Weksberg R, Scherer SW (2011) Hemizygous deletions on chromosome 1p21.3 involving the DPYD gene in individuals with autism spectrum disorder. Clin Genet 80(5):435–443. https://doi.org/10.1111/j.1399-0004.2010.01578.x
32. Willemsen MH, Valles A, Kirkels LA, Mastebroek M, Olde Loohuis N, Kos A, Wissink-Lindhout WM, de Brouwer AP, Nillesen WM, Pfundt R, Holder-Espinasse M, Vallee L, Andrieux J, Coppens-Hofman MC, Rensen H, Hamel BC, van Bokhoven H, Aschrafi A, Kleefstra T (2011) Chromosome 1p21.3 microdeletions comprising DPYD and MIR137 are associated with intellectual disability. J Med Genet 48(12):810–818. https://doi.org/10.1136/jmedgenet-2011-100294
33. Ripke S, O'Dushlaine C, Chambert K, Moran JL, Kahler AK, Akterin S, Bergen SE, Collins AL, Crowley JJ, Fromer M, Kim Y, Lee SH, Magnusson PK, Sanchez N, Stahl EA, Williams S, Wray NR, Xia K, Bettella F, Borglum AD, Bulik-Sullivan BK, Cormican P, Craddock N, de Leeuw C, Durmishi N, Gill M, Golimbet V, Hamshere ML, Holmans P, Hougaard DM, Kendler KS, Lin K, Morris DW, Mors O, Mortensen PB, Neale BM, O'Neill FA, Owen MJ, Milovancevic MP, Posthuma D, Powell J, Richards AL, Riley BP, Ruderfer D, Rujescu D, Sigurdsson E, Silagadze T, Smit AB, Stefansson H, Steinberg S, Suvisaari J, Tosato S, Verhage M, Walters JT, Multicenter Genetic Studies of Schizophrenia C, Levinson DF, Gejman PV, Kendler KS, Laurent C, Mowry BJ, O'Donovan MC, Owen MJ, Pulver AE, Riley BP, Schwab SG, Wildenauer DB, Dudbridge F, Holmans P, Shi J, Albus M, Alexander M, Campion D, Cohen D, Dikeos D, Duan J, Eichhammer P, Godard S, Hansen M, Lerer FB, Liang KY, Maier W, Mallet J, Nertney DA, Nestadt G, Norton N, O'Neill FA, Papadimitriou GN, Ribble R, Sanders AR, Silverman JM, Walsh D, Williams NM, Wormley B, Psychosis Endophenotypes International C, Arranz MJ, Bakker S, Bender S, Bramon E, Collier D, Crespo-Facorro B, Hall J, Iyegbe C, Jablensky A, Kahn RS, Kalaydjieva L, Lawrie S, Lewis CM, Lin K, Linszen DH, Mata I, McIntosh A, Murray RM, Ophoff RA, Powell J, Rujescu D, Van Os J, Walshe M, Weisbrod M, Wiersma D, Wellcome Trust Case Control C, Donnelly P, Barroso I, Blackwell JM, Bramon E, Brown MA, Casas JP, Corvin AP, Deloukas P, Duncanson A, Jankowski J, Markus HS, Mathew CG, Palmer CN, Plomin R, Rautanen A, Sawcer SJ, Trembath RC, Viswanathan AC, Wood NW, Spencer CC, Band G, Bellenguez C, Freeman C, Hellenthal G, Giannoulatou E, Pirinen M, Pearson RD, Strange A, Su Z, Vukcevic D, Donnelly P, Langford C, Hunt SE, Edkins S, Gwilliam R, Blackburn H, Bumpstead SJ, Dronov S, Gillman M, Gray E, Hammond N, Jayakumar A, McCann OT, Liddle J, Potter SC, Ravindrarajah R, Ricketts M, Tashakkori-Ghanbaria A, Waller MJ, Weston P, Widaa S, Whittaker P, Barroso I, Deloukas P, Mathew CG, Blackwell JM, Brown MA, Corvin AP, McCarthy MI, Spencer CC, Bramon E, Corvin AP, O'Donovan MC, Stefansson K, Scolnick E, Purcell S, McCarroll SA, Sklar P, Hultman CM, Sullivan PF (2013) Genome-wide association analysis identifies 13 new risk loci for schizophrenia. Nat Genet 45 (10):1150-1159. doi:10.1038/ng.2742
34. Siegert S, Seo J, Kwon EJ, Rudenko A, Cho S, Wang WY, Flood Z, Martorell AJ, Ericsson M, Mungenast AE, Tsai LH (2015) The schizophrenia risk gene product miR-137 alters presynaptic plasticity. Nat Neurosci 18(7):1008. https://doi.org/10.1038/nn.4023
35. Feingold M, Hall BD, Lacassie Y, Martinez-Frias ML (1997) Syndrome of microcephaly, facial and hand abnormalities, tracheoesophageal fistula, duodenal atresia, and developmental delay. Am J Med Genet 69(3):245–249
36. de Pontual L, Yao E, Callier P, Faivre L, Drouin V, Cariou S, Van Haeringen A, Genevieve D, Goldenberg A, Oufadem M, Manouvrier S, Munnich A, Vidigal JA, Vekemans M, Lyonnet

S, Henrion-Caude A, Ventura A, Amiel J (2011) Germline deletion of the miR-17 approximately 92 cluster causes skeletal and growth defects in humans. Nat Genet 43(10):1026–1030. https://doi.org/10.1038/ng.915
37. Pan WL, Chopp M, Fan B, Zhang R, Wang X, Hu J, Zhang XM, Zhang ZG, Liu XS (2019) Ablation of the microRNA-17-92 cluster in neural stem cells diminishes adult hippocampal neurogenesis and cognitive function. FASEB J 33(4):5257–5267. https://doi.org/10.1096/fj.201801019R
38. Jin J, Kim SN, Liu X, Zhang H, Zhang C, Seo JS, Kim Y, Sun T (2016) miR-17-92 cluster regulates adult hippocampal neurogenesis, anxiety, and depression. Cell Rep 16(6):1653–1663. https://doi.org/10.1016/j.celrep.2016.06.101
39. Fiori E, Babicola L, Andolina D, Coassin A, Pascucci T, Patella L, Han YC, Ventura A, Ventura R (2015) Neurobehavioral alterations in a genetic murine model of Feingold Syndrome 2. Behav Genet 45(5):547–559. https://doi.org/10.1007/s10519-015-9724-8
40. Toma C, Torrico B, Hervas A, Salgado M, Rueda I, Valdes-Mas R, Buitelaar JK, Rommelse N, Franke B, Freitag C, Reif A, Perez-Jurado LA, Battaglia A, Mazzone L, Bacchelli E, Puente XS, Cormand B (2015) Common and rare variants of microRNA genes in autism spectrum disorders. World J Biol Psychiatry 16(6):376–386. https://doi.org/10.3109/15622975.2015.1029518
41. Gaugler T, Klei L, Sanders SJ, Bodea CA, Goldberg AP, Lee AB, Mahajan M, Manaa D, Pawitan Y, Reichert J, Ripke S, Sandin S, Sklar P, Svantesson O, Reichenberg A, Hultman CM, Devlin B, Roeder K, Buxbaum JD (2014) Most genetic risk for autism resides with common variation. Nat Genet 46(8):881–885. https://doi.org/10.1038/ng.3039
42. Huguet G, Ey E, Bourgeron T (2013) The genetic landscapes of autism spectrum disorders. Annu Rev Genomics Hum Genet 14:191–213. https://doi.org/10.1146/annurev-genom-091212-153431
43. Basu SN, Kollu R, Banerjee-Basu S (2009) AutDB: a gene reference resource for autism research. Nucleic Acids Res 37(Database issue):D832–836. https://doi.org/10.1093/nar/gkn835
44. Mazzio EA, Soliman KF (2012) Basic concepts of epigenetics: impact of environmental signals on gene expression. Epigenetics 7(2):119–130. https://doi.org/10.4161/epi.7.2.18764
45. Abu-Elneel K, Liu T, Gazzaniga FS, Nishimura Y, Wall DP, Geschwind DH, Lao K, Kosik KS (2008) Heterogeneous dysregulation of microRNAs across the autism spectrum. Neurogenetics 9(3):153–161. https://doi.org/10.1007/s10048-008-0133-5
46. Ander BP, Barger N, Stamova B, Sharp FR, Schumann CM (2015) Atypical miRNA expression in temporal cortex associated with dysregulation of immune, cell cycle, and other pathways in autism spectrum disorders. Mol Autism 6:37. https://doi.org/10.1186/s13229-015-0029-9
47. Stamova B, Ander BP, Barger N, Sharp FR, Schumann CM (2015) Specific regional and age-related small noncoding RNA expression patterns within superior temporal gyrus of typical human brains are less distinct in autism brains. J Child Neurol 30(14):1930–1946. https://doi.org/10.1177/0883073815602067
48. Mor M, Nardone S, Sams DS, Elliott E (2015) Hypomethylation of miR-142 promoter and upregulation of microRNAs that target the oxytocin receptor gene in the autism prefrontal cortex. Mol Autism 6:46. https://doi.org/10.1186/s13229-015-0040-1
49. Huang F, Long Z, Chen Z, Li J, Hu Z, Qiu R, Zhuang W, Tang B, Xia K, Jiang H (2015) Investigation of gene regulatory networks associated with autism spectrum disorder based on MiRNA expression in China. PLoS ONE 10(6):e0129052. https://doi.org/10.1371/journal.pone.0129052
50. Mundalil Vasu M, Anitha A, Thanseem I, Suzuki K, Yamada K, Takahashi T, Wakuda T, Iwata K, Tsujii M, Sugiyama T, Mori N (2014) Serum microRNA profiles in children with autism. Mol Autism 5:40. https://doi.org/10.1186/2040-2392-5-40
51. Ghahramani Seno MM, Hu P, Gwadry FG, Pinto D, Marshall CR, Casallo G, Scherer SW (2011) Gene and miRNA expression profiles in autism spectrum disorders. Brain Res 1380:85–97. https://doi.org/10.1016/j.brainres.2010.09.046
52. Sarachana T, Zhou R, Chen G, Manji HK, Hu VW (2010) Investigation of post-transcriptional gene regulatory networks associated with autism spectrum disorders by microRNA expression profiling of lymphoblastoid cell lines. Genome Med 2(4):23. https://doi.org/10.1186/gm144
53. Talebizadeh Z, Butler MG, Theodoro MF (2008) Feasibility and relevance of examining lymphoblastoid cell lines to study role of microRNAs in autism. Autism Res 1(4):240–250. https://doi.org/10.1002/aur.33
54. Nguyen LS, Lepleux M, Makhlouf M, Martin C, Fregeac J, Siquier-Pernet K, Philippe A, Feron F, Gepner B, Rougeulle C, Humeau Y, Colleaux L (2016) Profiling olfactory stem cells from living patients identifies miRNAs relevant for autism pathophysiology. Mol Autism 7:1. https://doi.org/10.1186/s13229-015-0064-6
55. Ziats MN, Rennert OM (2014) Identification of differentially expressed microRNAs across the developing human brain. Mol Psychiatry 19(7):848–852. https://doi.org/10.1038/mp.2013.93
56. Yu D, Jiao XQ, Cao T, Huang FS (2018) Serum miRNA expression profiling reveals miR-486-3p may play a significant role in the development of autism by targeting ARID1B. NeuroReport 29(17):1431–1436. https://doi.org/10.1097/Wnr.0000000000001107
57. Hicks SD, Carpenter RL, Wagner KE, Pauley R, Barros M, Tierney-Aves C, Barns S, Greene CD, Middleton FA (2020) Saliva MicroRNA differentiates children with autism from peers with typical and atypical development. J Am Acad Child Adolesc Psychiatry 59(2):296–308. https://doi.org/10.1016/j.jaac.2019.03.017
58. Jovicic A, Roshan R, Moisoi N, Pradervand S, Moser R, Pillai B, Luthi-Carter R (2013) Comprehensive expression analyses of neural cell-type-specific miRNAs identify new determinants of the specification and maintenance of neuronal phenotypes. J Neurosci 33(12):5127–5137. https://doi.org/10.1523/JNEUROSCI.0600-12.2013
59. Nguyen LS, Fregeac J, Bole-Feysot C, Cagnard N, Iyer A, Anink J, Aronica E, Alibeu O, Nitschke P, Colleaux L (2018) Role of miR-146a in neural stem cell differentiation and neural lineage determination: relevance for neurodevelopmental disorders. Mol Autism 9:38. https://doi.org/10.1186/s13229-018-0219-3
60. Chen YL, Shen CK (2013) Modulation of mGluR-dependent MAP1B translation and AMPA receptor endocytosis by microRNA miR-146a-5p. J Neurosci 33(21):9013–9020. https://doi.org/10.1523/JNEUROSCI.5210-12.2013
61. Hicks SD, Rajan AT, Wagner KE, Barns S, Carpenter RL, Middleton FA (2018) Validation of a salivary RNA test for childhood autism spectrum disorder. Front Genet 9:534. https://doi.org/10.3389/fgene.2018.00534
62. Aronica E, Fluiter K, Iyer A, Zurolo E, Vreijling J, van Vliet EA, Baayen JC, Gorter JA (2010) Expression pattern of miR-146a, an inflammation-associated microRNA, in experimental and human temporal lobe epilepsy. Eur J Neurosci 31(6):1100–1107. https://doi.org/10.1111/j.1460-9568.2010.07122.x
63. Iyer A, Zurolo E, Prabowo A, Fluiter K, Spliet WG, van Rijen PC, Gorter JA, Aronica E (2012) MicroRNA-146a: a key regulator of astrocyte-mediated inflammatory response. PLoS ONE 7(9):e44789. https://doi.org/10.1371/journal.pone.0044789
64. Hsu PK, Xu B, Mukai J, Karayiorgou M, Gogos JA (2015) The BDNF Val66Met variant affects gene expression through

miR-146b. Neurobiol Dis 77:228–237. https://doi.org/10.1016/j.nbd.2015.03.004
65. Guo Q, Zhang J, Li J, Zou L, Zhang J, Xie Z, Fu X, Jiang S, Chen G, Jia Q, Li F, Wan Y, Wu Y (2013) Forced miR-146a expression causes autoimmune lymphoproliferative syndrome in mice via downregulation of Fas in germinal center B cells. Blood 121(24):4875–4883. https://doi.org/10.1182/blood-2012-08-452425
66. Boldin MP, Taganov KD, Rao DS, Yang L, Zhao JL, Kalwani M, Garcia-Flores Y, Luong M, Devrekanli A, Xu J, Sun G, Tay J, Linsley PS, Baltimore D (2011) miR-146a is a significant brake on autoimmunity, myeloproliferation, and cancer in mice. J Exp Med 208(6):1189–1201. https://doi.org/10.1084/jem.20101823
67. Hannenhalli S, Kaestner KH (2009) The evolution of Fox genes and their role in development and disease. Nat Rev Genet 10(4):233–240. https://doi.org/10.1038/nrg2523
68. Clovis YM, Enard W, Marinaro F, Huttner WB, De Pietri TD (2012) Convergent repression of Foxp2 3'UTR by miR-9 and miR-132 in embryonic mouse neocortex: implications for radial migration of neurons. Development 139(18):3332–3342. https://doi.org/10.1242/dev.078063
69. Tognini P, Putignano E, Coatti A, Pizzorusso T (2011) Experience-dependent expression of miR-132 regulates ocular dominance plasticity. Nat Neurosci 14(10):1237–1239. https://doi.org/10.1038/nn.2920
70. Hansen KF, Sakamoto K, Wayman GA, Impey S, Obrietan K (2010) Transgenic miR132 alters neuronal spine density and impairs novel object recognition memory. PLoS ONE 5(11):e15497. https://doi.org/10.1371/journal.pone.0015497
71. Cheng TL, Qiu Z (2014) MeCP2: multifaceted roles in gene regulation and neural development. Neurosci Bull 30(4):601–609. https://doi.org/10.1007/s12264-014-1452-6
72. Cheng TL, Wang Z, Liao Q, Zhu Y, Zhou WH, Xu W, Qiu Z (2014) MeCP2 suppresses nuclear microRNA processing and dendritic growth by regulating the DGCR8/Drosha complex. Dev Cell 28(5):547–560. https://doi.org/10.1016/j.devcel.2014.01.032
73. Lyu JW, Yuan B, Cheng TL, Qiu ZL, Zhou WH (2016) Reciprocal regulation of autism-related genes MeCP2 and PTEN via microRNAs. Sci Rep 6:20392. https://doi.org/10.1038/srep20392
74. Han K, Gennarino VA, Lee Y, Pang K, Hashimoto-Torii K, Choufani S, Raju CS, Oldham MC, Weksberg R, Rakic P, Liu Z, Zoghbi HY (2013) Human-specific regulation of MeCP2 levels in fetal brains by microRNA miR-483-5p. Genes Dev 27(5):485–490. https://doi.org/10.1101/gad.207456.112
75. Jacquemont ML, Sanlaville D, Redon R, Raoul O, Cormier-Daire V, Lyonnet S, Amiel J, Le Merrer M, Heron D, de Blois MC, Prieur M, Vekemans M, Carter NP, Munnich A, Colleaux L, Philippe A (2006) Array-based comparative genomic hybridisation identifies high frequency of cryptic chromosomal rearrangements in patients with syndromic autism spectrum disorders. J Med Genet 43(11):843–849. https://doi.org/10.1136/jmg.2006.043166
76. Sebat J, Lakshmi B, Malhotra D, Troge J, Lese-Martin C, Walsh T, Yamrom B, Yoon S, Krasnitz A, Kendall J, Leotta A, Pai D, Zhang R, Lee YH, Hicks J, Spence SJ, Lee AT, Puura K, Lehtimaki T, Ledbetter D, Gregersen PK, Bregman J, Sutcliffe JS, Jobanputra V, Chung W, Warburton D, King MC, Skuse D, Geschwind DH, Gilliam TC, Ye K, Wigler M (2007) Strong association of de novo copy number mutations with autism. Science 316(5823):445–449. https://doi.org/10.1126/science.1138659
77. Marrale M, Albanese NN, Cali F, Romano V (2014) Assessing the impact of copy number variants on miRNA genes in autism by Monte Carlo simulation. PLoS ONE 9(3):e90947. https://doi.org/10.1371/journal.pone.0090947
78. Iwai N, Naraba H (2005) Polymorphisms in human pre-miRNAs. Biochem Biophys Res Commun 331(4):1439–1444. https://doi.org/10.1016/j.bbrc.2005.04.051
79. Saunders MA, Liang H, Li WH (2007) Human polymorphism at microRNAs and microRNA target sites. Proc Natl Acad Sci USA 104(9):3300–3305. https://doi.org/10.1073/pnas.0611347104
80. Li L, Meng T, Jia Z, Zhu G, Shi B (2010) Single nucleotide polymorphism associated with nonsyndromic cleft palate influences the processing of miR-140. Am J Med Genet A 152A(4):856–862. https://doi.org/10.1002/ajmg.a.33236
81. Sun G, Yan J, Noltner K, Feng J, Li H, Sarkis DA, Sommer SS, Rossi JJ (2009) SNPs in human miRNA genes affect biogenesis and function. RNA 15(9):1640–1651. https://doi.org/10.1261/rna.1560209
82. Dietert RR, Dietert JM, Dewitt JC (2011) Environmental risk factors for autism. Emerg Health Threats J 4:7111. https://doi.org/10.3402/ehtj.v4i0.7111
83. Lancon A, Michaille JJ, Latruffe N (2013) Effects of dietary phytophenols on the expression of microRNAs involved in mammalian cell homeostasis. J Sci Food Agric 93(13):3155–3164. https://doi.org/10.1002/jsfa.6228
84. Li Y, Kong D, Wang Z, Sarkar FH (2010) Regulation of microRNAs by natural agents: an emerging field in chemoprevention and chemotherapy research. Pharm Res 27(6):1027–1041. https://doi.org/10.1007/s11095-010-0105-y
85. Weldon BA, Shubin SP, Smith MN, Workman T, Artemenko A, Griffith WC, Thompson B, Faustman EM (2016) Urinary microRNAs as potential biomarkers of pesticide exposure. Toxicol Appl Pharmacol 312:19–25. https://doi.org/10.1016/j.taap.2016.01.018
86. Venault P, Chapouthier G (2007) Plasticity and anxiety. Neural Plast 2007:75617. https://doi.org/10.1155/2007/75617
87. Osuch EA, Ketter TA, Kimbrell TA, George MS, Benson BE, Willis MW, Herscovitch P, Post RM (2000) Regional cerebral metabolism associated with anxiety symptoms in affective disorder patients. Biol Psychiatry 48(10):1020–1023. https://doi.org/10.1016/s0006-3223(00)00920-3
88. Davidson RJ, Abercrombie H, Nitschke JB, Putnam K (1999) Regional brain function, emotion and disorders of emotion. Curr Opin Neurobiol 9(2):228–234
89. McNaughton N (1997) Cognitive dysfunction resulting from hippocampal hyperactivity—a possible cause of anxiety disorder? Pharmacol Biochem Behav 56(4):603–611. https://doi.org/10.1016/s0091-3057(96)00419-4
90. Haramati S, Navon I, Issler O, Ezra-Nevo G, Gil S, Zwang R, Hornstein E, Chen A (2011) MicroRNA as repressors of stress-induced anxiety: the case of amygdalar miR-34. J Neurosci 31(40):14191–14203. https://doi.org/10.1523/JNEUROSCI.1673-11.2011
91. Andolina D, Di Segni M, Bisicchia E, D'Alessandro F, Cestari V, Ventura A, Concepcion C, Puglisi-Allegra S, Ventura R (2016) Effects of lack of microRNA-34 on the neural circuitry underlying the stress response and anxiety. Neuropharmacology 107:305–316. https://doi.org/10.1016/j.neuropharm.2016.03.044
92. Zhu J, Chen Z, Tian J, Meng Z, Ju M, Wu G, Tian Z (2017) miR-34b attenuates trauma-induced anxiety-like behavior by targeting CRHR1. Int J Mol Med 40(1):90–100. https://doi.org/10.3892/ijmm.2017.2981
93. Aten S, Page CE, Kalidindi A, Wheaton K, Niraula A, Godbout JP, Hoyt KR, Obrietan K (2019) miR-132/212 is induced by stress and its dysregulation triggers anxiety-related behavior.

Neuropharmacology 144:256–270. https://doi.org/10.1016/j.neuropharm.2018.10.020
94. Cohen JL, Jackson NL, Ballestas ME, Webb WM, Lubin FD, Clinton SM (2017) Amygdalar expression of the microRNA miR-101a and its target Ezh2 contribute to rodent anxiety-like behaviour. Eur J Neurosci 46(7):2241–2252. https://doi.org/10.1111/ejn.13624
95. Fonken LK, Gaudet AD, Gaier KR, Nelson RJ, Popovich PG (2016) MicroRNA-155 deletion reduces anxiety- and depressive-like behaviors in mice. Psychoneuroendocrinology 63:362–369. https://doi.org/10.1016/j.psyneuen.2015.10.019
96. Issler O, Haramati S, Paul ED, Maeno H, Navon I, Zwang R, Gil S, Mayberg HS, Dunlop BW, Menke A, Awatramani R, Binder EB, Deneris ES, Lowry CA, Chen A (2014) MicroRNA 135 is essential for chronic stress resiliency, antidepressant efficacy, and intact serotonergic activity. Neuron 83(2):344–360. https://doi.org/10.1016/j.neuron.2014.05.042
97. Mannironi C, Biundo A, Rajendran S, De Vito F, Saba L, Caioli S, Zona C, Ciotti T, Caristi S, Perlas E, Del Vecchio G, Bozzoni I, Rinaldi A, Mele A, Presutti C (2018) miR-135a regulates synaptic transmission and anxiety-like behavior in amygdala. Mol Neurobiol 55(4):3301–3315. https://doi.org/10.1007/s12035-017-0564-9
98. Marty V, Labialle S, Bortolin-Cavaille ML, Ferreira De Medeiros G, Moisan MP, Florian C, Cavaille J (2016) Deletion of the miR-379/miR-410 gene cluster at the imprinted Dlk1-Dio3 locus enhances anxiety-related behaviour. Hum Mol Genet 25(4):728–739. https://doi.org/10.1093/hmg/ddv510
99. Parsons MJ, Grimm CH, Paya-Cano JL, Sugden K, Nietfeld W, Lehrach H, Schalkwyk LC (2008) Using hippocampal microRNA expression differences between mouse inbred strains to characterise miRNA function. Mamm Genome 19(7–8):552–560. https://doi.org/10.1007/s00335-008-9116-y
100. Rinaldi A, Vincenti S, De Vito F, Bozzoni I, Oliverio A, Presutti C, Fragapane P, Mele A (2010) Stress induces region specific alterations in microRNAs expression in mice. Behav Brain Res 208(1):265–269. https://doi.org/10.1016/j.bbr.2009.11.012
101. Uchida S, Nishida A, Hara K, Kamemoto T, Suetsugi M, Fujimoto M, Watanuki T, Wakabayashi Y, Otsuki K, McEwen BS, Watanabe Y (2008) Characterization of the vulnerability to repeated stress in Fischer 344 rats: possible involvement of microRNA-mediated down-regulation of the glucocorticoid receptor. Eur J Neurosci 27(9):2250–2261. https://doi.org/10.1111/j.1460-9568.2008.06218.x
102. Babenko O, Golubov A, Ilnytskyy Y, Kovalchuk I, Metz GA (2012) Genomic and epigenomic responses to chronic stress involve miRNA-mediated programming. PLoS ONE 7(1):e29441. https://doi.org/10.1371/journal.pone.0029441
103. Bradesi S, Karagiannides I, Bakirtzi K, Joshi SM, Koukos G, Iliopoulos D, Pothoulakis C, Mayer EA (2015) Identification of spinal cord microRNA and gene signatures in a model of chronic stress-induced visceral hyperalgesia in rat. PLoS ONE 10(7):e0130938. https://doi.org/10.1371/journal.pone.0130938
104. Zhang Y, Wang Y, Wang L, Bai M, Zhang X, Zhu X (2015) Dopamine receptor D2 and associated microRNAs are involved in stress susceptibility and resistance to escitalopram treatment. Int J Neuropsychopharmacol. https://doi.org/10.1093/ijnp/pyv025
105. Meerson A, Cacheaux L, Goosens KA, Sapolsky RM, Soreq H, Kaufer D (2010) Changes in brain MicroRNAs contribute to cholinergic stress reactions. J Mol Neurosci 40(1–2):47–55. https://doi.org/10.1007/s12031-009-9252-1
106. Nadorp B, Soreq H (2014) Predicted overlapping microRNA regulators of acetylcholine packaging and degradation in neuroinflammation-related disorders. Front Mol Neurosci 7:9. https://doi.org/10.3389/fnmol.2014.00009
107. Gapp K, Jawaid A, Sarkies P, Bohacek J, Pelczar P, Prados J, Farinelli L, Miska E, Mansuy IM (2014) Implication of sperm RNAs in transgenerational inheritance of the effects of early trauma in mice. Nat Neurosci 17(5):667–669. https://doi.org/10.1038/nn.3695
108. Gapp K, van Steenwyk G, Germain PL, Matsushima W, Rudolph KLM, Manuella F, Roszkowski M, Vernaz G, Ghosh T, Pelczar P, Mansuy IM, Miska EA (2018) Alterations in sperm long RNA contribute to the epigenetic inheritance of the effects of postnatal trauma. Mol Psychiatry. https://doi.org/10.1038/s41380-018-0271-6
109. Benaroya-Milshtein N, Hollander N, Apter A, Kukulansky T, Raz N, Wilf A, Yaniv I, Pick CG (2004) Environmental enrichment in mice decreases anxiety, attenuates stress responses and enhances natural killer cell activity. Eur J Neurosci 20(5):1341–1347. https://doi.org/10.1111/j.1460-9568.2004.03587.x
110. Brenes JC, Lackinger M, Hoglinger GU, Schratt G, Schwarting RK, Wohr M (2016) Differential effects of social and physical environmental enrichment on brain plasticity, cognition, and ultrasonic communication in rats. J Comp Neurol 524(8):1586–1607. https://doi.org/10.1002/cne.23842
111. Hohoff C, Weber H, Richter J, Domschke K, Zwanzger PM, Ohrmann P, Bauer J, Suslow T, Kugel H, Baumann C, Klauke B, Jacob CP, Fritze J, Bandelow B, Gloster AT, Gerlach AL, Kircher T, Lang T, Alpers GW, Strohle A, Fehm L, Wittchen HU, Arolt V, Pauli P, Hamm A, Reif A, Deckert J (2015) RGS2 genetic variation: association analysis with panic disorder and dimensional as well as intermediate phenotypes of anxiety. Am J Med Genet B Neuropsychiatr Genet 168B(3):211–222. https://doi.org/10.1002/ajmg.b.32299
112. Leygraf A, Hohoff C, Freitag C, Willis-Owen SA, Krakowitzky P, Fritze J, Franke P, Bandelow B, Fimmers R, Flint J, Deckert J (2006) Rgs 2 gene polymorphisms as modulators of anxiety in humans? J Neural Transm (Vienna) 113(12):1921–1925. https://doi.org/10.1007/s00702-006-0484-8
113. Hommers L, Raab A, Bohl A, Weber H, Scholz CJ, Erhardt A, Binder E, Arolt V, Gerlach A, Gloster A, Kalisch R, Kircher T, Lonsdorf T, Strohle A, Zwanzger P, Mattheisen M, Cichon S, Lesch KP, Domschke K, Reif A, Lohse MJ, Deckert J (2015) MicroRNA hsa-miR-4717-5p regulates RGS2 and may be a risk factor for anxiety-related traits. Am J Med Genet B Neuropsychiatr Genet 168B(4):296–306. https://doi.org/10.1002/ajmg.b.32312
114. Muinos-Gimeno M, Espinosa-Parrilla Y, Guidi M, Kagerbauer B, Sipila T, Maron E, Pettai K, Kananen L, Navines R, Martin-Santos R, Gratacos M, Metspalu A, Hovatta I, Estivill X (2011) Human microRNAs miR-22, miR-138-2, miR-148a, and miR-488 are associated with panic disorder and regulate several anxiety candidate genes and related pathways. Biol Psychiatry 69(6):526–533. https://doi.org/10.1016/j.biopsych.2010.10.010
115. Wang X, Sundquist K, Hedelius A, Palmer K, Memon AA, Sundquist J (2015) Circulating microRNA-144-5p is associated with depressive disorders. Clin Epigenet 7:69. https://doi.org/10.1186/s13148-015-0099-8
116. Rajman M, Schratt G (2017) MicroRNAs in neural development: from master regulators to fine-tuners. Development 144(13):2310–2322. https://doi.org/10.1242/dev.144337
117. Issler O, Chen A (2015) Determining the role of microRNAs in psychiatric disorders. Nat Rev Neurosci 16(4):201–212. https://doi.org/10.1038/nrn3879